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TEMPERATURE AND SOIL-MOISTURE RELATIONS OF FUSARIUM OXYSPORUM VAR. MEDICAGINIS[1]

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INTRODUCTION

Recent investigations have shown that a disease caused by *Fusarium oxysporum* var. *medicaginis* is present in alfalfa in northeastern Mississippi (*9*, *10*).[2] Although the distribution of this disease may be quite general, it is definitely known to exist only in that State. It seems very probable, however, that it will eventually be found to occur at least in other Southern States. The seemingly limited distribution of the disease suggested the possibility of a striking reaction to some environmental factor such as temperature or moisture. This led to a study of the response of the causal organism to temperature when growing in pure culture, and of the influence of soil temperature and moisture on infection. The results of these investigations form the basis of this paper.

METHODS

The strain of *Fusarium oxysporum* var. *medicaginis* used in these studies was a pure line of the original isolation and the same as that from which the description was made (*10*). The effect of temperature on the growth of the mycelium was determined by placing a loop of a suspension of the spores of the fungus on agar in the center of Petri dishes and measuring the rate of growth. The agar was 2 per cent bactonutrient agar plus 2 per cent dextrose, a medium on which the fungus is known to grow luxuriantly. The spores used were obtained largely from sporodochia of a 60-day-old culture growing on potato agar. As soon as the plates were prepared they were placed in incubators held at the desired temperatures, five dishes being used at each temperature.

The effect of soil temperature on infection by *Fusarium oxysporum* var. *medicaginis* was determined by growing plants in temperature tanks similar to those described by Jones, Johnson, and Dickson (*6*). Each tank was provided with a thermostatic control so that the soil temperature usually did not vary more than 1° or 2°. The temperature of the coldest tank, which ranged from 16° to 18° C., was maintained by running water continuously from the tap through the tank. The soil was prepared by mixing 3 parts of good river-bottom soil

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[2] Reference is made by number (italic) to "Literature Cited," p. 103.

with 1 part of sand and 1 part of fine, well-rotted stable manure. Before the mixture was used it was placed in shallow flats and steamed for four hours without pressure. The moisture-holding capacity of the soil was determined at the beginning of the experiment, and the moisture content was adjusted as desired. The moisture content was maintained by weighing the pots frequently and by adding water as needed. The water was added in each case either to the surface of the soil or through a thistle tube inserted through the hole in the bottom of a 2-inch unglazed flower pot inverted in the soil slightly below the center of the container. The soil moisture could not be kept uniform throughout the pots in this manner, but no better method was known. The surface of the soil was covered with granulated cork to retard evaporation. The tops of the plants were subjected to ordinary greenhouse temperatures, which ranged from 10° to 30° and averaged about 20°.

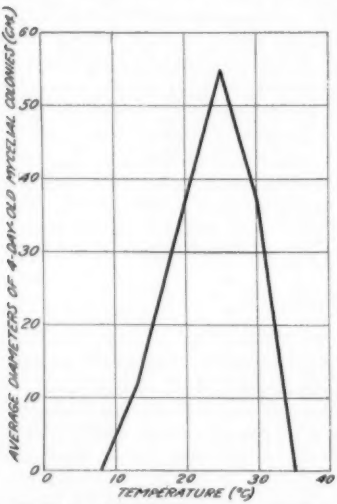

FIGURE 1.—Comparative mycelial growths made by *Fusarium oxysporum* var. *medicaginis* in four days at different temperatures

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

The temperatures to which the fungus in the Petri dishes was exposed ranged from 3° to 38° C. The comparative rate of mycelial growth in four days at various temperatures is illustrated by the curve in Figure 1. It is clear from this curve that the optimum temperature for mycelial growth lies not far from 25°. There was some growth at 13°, but none at 8°. No macroscopically visible growth was made at 35°. The thermal death point of the fungus lies between 38° and 39°.

The spores germinated, but they made little hyphal growth in three days at a temperature of 37° to 38°. In four days at 8° the germ tubes were about three to five times the length of the spores. None had germinated at 3° in four days, but some of the spores were swollen, indicating that germination would have taken place if it had been practicable to continue the experiment longer.

It is generally known that the cardinal temperatures for the growth of fungi differ somewhat with the strains as well as with the substrata upon which they are growing. Edson and Shapovalov (*2*) showed that the optimum for the growth of the strain of *Fusarium oxysporum* studied by them when growing on potato agar without sugar was 30° C. Goss (*3*), on the other hand, found that the two strains of *F. oxysporum* studied by him had their optimum temperatures for growth at 25° and 30°, respectively, when growing on hard potato agar. Link (*7*) gave the optimum temperature for the growth of *F. oxysporum* on liquid glucose media as about 30°. Both Goss

and Link gave the maximum temperature for the strains with which they worked as near 40°. This temperature agrees very closely with that for *F. oxysporum* var. *medicaginis*.

According to Haskell (*4*), the optimum temperature for *Fusarium oxysporum* growing in liquid media was 26° to 32° and the maximum was 40° C. Johnson (*5*) and Massey (*8*) stated that the optimum temperatures of the varieties of *F. oxysporum* from tobacco and gladiolus were 28° to 30° and 27.5°, respectively.

It seems fairly well established, therefore, that the optimum temperature for the various strains and varieties of *Fusarium oxysporum* lies somewhere between 25° and 30° C.

Edson and Shapovalov (*2*) pointed out that, of the fungi with which they worked, *Fusarium coeruleum* and *Verticillium alboatrum* from the northern section of the country had lower maximum and optimum temperatures than *F. radicicola* and *F. oxysporum*, parasites from the South. There does not seem to be such a correlation between the geographical source of *F. oxysporum* var. *medicaginis* and its cardinal temperatures, since it is a southern fungus and yet has an optimum for growth as low as or lower than that of any of the strains or varieties of *F. oxysporum* listed above, some of which are from the North. Furthermore, it is quite evident that the cardinal temperatures for *F. oxysporum* var. *medicaginis* when growing in pure culture are such that temperature is not a limiting factor in its distribution in this country.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON INFECTION

Soil moisture, as well as temperature, is often a limiting factor in the prevalence and spread of a disease. To determine whether the amount of Fusarium wilt of alfalfa would probably be very greatly influenced by soil moisture, and therefore would be restricted largely to sections of high or low rainfall, two different soil moistures were used in the temperature-tank series run during the winter of 1926–27.

The soil moisture in one half of the cans of each tank was held at 35 per cent and in the other half at 55 per cent of the water-holding capacity of the soil. The alfalfa plants used were of the Kansas Common variety grown from seed sown in the field in August, 1926. The plants were brought to the greenhouse on February 1, 1927, when the experiment was begun, and 10 plants were set in each can. The plants in one of the cans at each moisture and temperature were inoculated by inserting hyphae beneath the bark of the taproot just below the crown, and those of another were inoculated by pouring about the plants 100 c. c. of a very heavy spore suspension. The two remaining pots at each moisture and temperature were held uninoculated as controls, the plants in one being left uninjured and those in the other being injured like those in the wounded-inoculated lot. This made four pots of each soil moisture at each temperature. The temperatures used were 17°, 21°, 25°, 30°, and 35° C. A record of the temperatures was made two or three times a day throughout the duration of the experiment, and the figures given above are the averages of those taken.

When a plant became infected and the disease had developed sufficiently so that there was little doubt as to its origin, the plant

was removed and isolations were made to confirm the diagnosis. It was necessary to remove the infected plants and maintain a record of them, since the disease developed so slowly and irregularly that some plants would have entirely disintegrated before the termination of the experiment (May 9, 1927). The first evidence of infection appeared on February 26 in the can with 55 per cent soil moisture held at 25° C. and inoculated by inserting the fungus in a wound. Subsequently, infected plants appeared from time to time. The percentages of infection at each temperature as influenced by the method of inoculation and soil moisture are presented graphically in Figures 2 and 3.

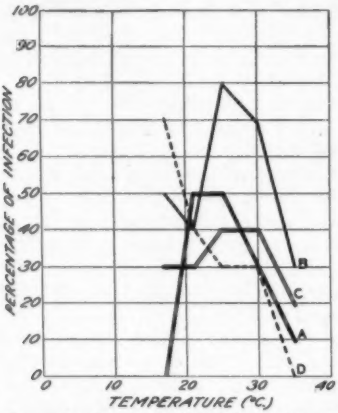

FIGURE 2.—Effect of method of inoculation on percentage of Fusarium infection of alfalfa when growing under different soil-moisture conditions: A and B, Inoculum inserted in roots through wounds: A, At 35 per cent soil moisture; B, at 55 per cent soil moisture; C and D, inoculated by pouring a spore suspension about the roots; C, at 35 per cent soil moisture; D, at 55 per cent soil moisture

An examination of the curves in Figure 2 shows that the percentage of infection was greatest when the inoculum was inserted into the root through wounds in the plants growing in a soil with a moisture content equal to 55 per cent of its water-holding capacity. In this case the optimum for infection was 25° C. A comparatively high percentage of plants also became diseased at 17°, the lowest temperature tried. The high percentage of infection obtained by inserting the inoculum in the host growing in a soil containing 55 per cent water was in marked contrast to that obtained by treating the plants with a spore suspension only. The one notable exception was at 17°. At this temperature 70 per cent of the plants became infected. At 35 per cent soil moisture the difference in the amount of infection was also in favor of the wounding method of inoculation at some temperatures but not at others. For example, at 17°, 30°, and 35° more disease resulted in the unwounded plants, while the reverse was true at 21° and 25°. Judging from these curves, the optimum temperature is not very sharp, ranging from 17° to 30°, depending on conditions. It seems probable that there are too many factors considered in these instances to bring out clearly the effect of temperature.

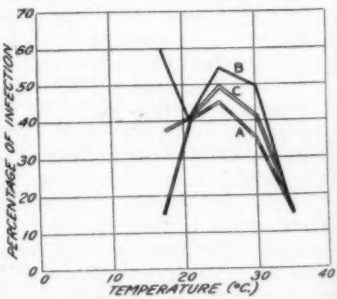

FIGURE 3.—Effect of soil moisture and temperature on percentage of Fusarium infection of alfalfa regardless of the method of inoculation: A, At 35 per cent soil moisture; B, at 55 per cent soil moisture; C, average at both soil moistures

Figure 3 shows the effect of soil moisture and temperature on infection regardless of the method of inoculation, and the average percentage

of infection at each temperature when the other factors are disregarded. These curves have two points in common, namely, their intersections at 21° and 35° C.

They all show an optimum at 25°, although the curve illustrating the infection at 55 per cent soil moisture shows another and even higher optimum at 17°. No explanation for the high percentage of infection at 17° in this case can be seen. It did not occur in the experiment conducted in 1927–28, as is shown in Figure 4. It is clear that 55 per cent soil moisture is more favorable for infection than 35 per cent.

In order to verify the results of the foregoing experiment and to determine whether the influence of temperature varies with the variety of alfalfa used, a similar experiment was run during the winter of 1927–28. The general plan of this test was somewhat different from that run the previous year. Since the 55 per cent soil moisture proved to be the more favorable of the two tried in 1926–27, this one only was used in 1927–28. Approximately the same temperatures were employed as in the previous year. The plants used were of the Hairy Peruvian, an extremely nonhardy variety, and Grimm, one of the hardiest varieties. It was thought that perhaps the disease might react differently on these two varieties, which themselves respond so differently to temperature.

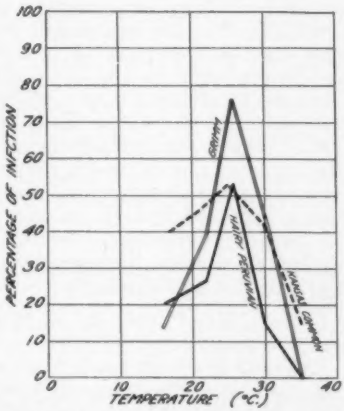

FIGURE 4.—Percentages of infection by *Fusarium oxysporum* var. *medicaginis* in Grimm, Kansas Common, and Hairy Peruvian alfalfas growing in soil at different temperatures and at 55 per cent soil moisture

The soil was made up to the desired moisture content, the seeds were inoculated with *Bacillus radicicola*, and one-half of the pots were planted with each variety. After the plants had grown for 39 days, four pots of each variety were placed in each of the five tanks. Three of the pots of each lot were then inoculated by pouring a heavy spore suspension about the plants. The fourth pot was held uninoculated as a control in each case. The plants were inoculated on November 12, 1927, and the first infection became evident in a Grimm plant on December 1, in the 25° C. tank. On December 12, 10 plants of the Grimm and one of the Hairy Peruvian variety in the 25° tank were diseased. By December 23 a total of 16 plants of the Grimm variety and four of the Hairy Peruvian had become infected at 25°, the optimum temperature. This showed that Grimm was more susceptible, at least at that temperature. Figure 4 shows the percentage of plants of each variety that finally became infected at the different temperatures. Grimm became infected not only earlier but also more heavily at all but the lowest temperature. No disease developed at 35° in either variety. As in the previous experiment, the optimum for infection was near 25°, which coincides also with the optimum for the growth of the fungus in pure culture. The curve obtained the

previous year when Kansas Common alfalfa was used is also included in Figure 4 for comparison. Kansas Common and Hairy Peruvian gave the same percentage at the optimum temperature, while Grimm was considerably more susceptible under the conditions of this experiment.

These data show that alfalfa may be attacked by *Fusarium oxysporum* var. *medicaginis* over a range of temperatures from 16° to 35° C. The optimum lies somewhere near 25° and the maximum near 35°. The optimum coincides with that for mycelial growth. The minimum temperature for infection was not determined, but it probably is lower than the growing point of alfalfa.

Perhaps a few words about the growth of the different varieties of alfalfa used under the conditions of these experiments would not be out of place. The Kansas Common variety grew more vigorously at a soil moisture of 55 per cent than of 35 per cent. The optimum temperature for the growth of all three varieties was between 20° and 30° C., falling somewhere near 25°. It was noticeable in most instances that the optimum for growth of the alfalfa plants was also the most suitable for their infection by *Fusarium oxysporum* var. *medicaginis* and for the development of the wilt disease. Grimm and Kansas Common made considerably less growth at 16° and 17°, respectively, and at 35° than at temperatures between 20° and 30°. On the other hand, Hairy Peruvian showed a decidedly greater range for vigorous development, for it grew very well throughout the range of temperatures used, as nearly as could be determined by observation alone, although it did seem to show slightly better growth at 25° than at the other temperatures tried. The fact that Peruvian alfalfa will grow over a wider temperature range than the common alfalfa was pointed out by Brand (*1*).

SUMMARY

A study has been made to determine the effect of temperature upon the growth of *Fusarium oxysporum* var. *medicaginis* in pure culture and upon its ability to attack the alfalfa plant.

The optimum temperature for mycelial growth of this fungus in pure culture is near 25° C., and the maximum is about 37° or 38°. The minimum was not determined, but it lies somewhere below 3°. In general, it may be said that the cardinal temperatures for this fungus do not differ greatly from those for *Fusarium oxysporum*. Although *F. oxysporum* var. *medicaginis* is known to occur only in the South, there appears to be no reason, so far as its cardinal temperatures are concerned, why it should not be widely distributed throughout the country.

Experiments with Kansas Common alfalfa grown in soil at definite soil temperatures and moistures showed that 55 per cent soil moisture was more favorable than 35 per cent for its growth as well as for its infection with *Fusarium oxysporum* var. *medicaginis*. Infection was more certain when the inoculum was inserted beneath the bark of the taproot than when it was added to the soil about the roots. The optimum temperature for infection lies near 25° C., and the disease develops, although poorly, at 35°. The lowest temperature tried was 17°, at which considerable infection resulted. Thus the optimum for infection agrees with that for mycelial growth. Infection takes place

over so wide a range of temperature that this can not be a factor in limiting the distribution of this fungus in this country.

Grimm and Hairy Peruvian alfalfa are also susceptible to the wilt disease, showing about the same optimum temperature for infection. Grimm was slightly more heavily infected than Hairy Peruvian in these experiments.

Hairy Peruvian alfalfa showed a somewhat better growth at the extreme temperatures than did Grimm or Kansas Common.

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A PHYSIOLOGICAL METHOD OF DISTINGUISHING CRONARTIUM RIBICOLA AND C. OCCIDENTALE IN THE UREDINIAL STAGES[1]

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INTRODUCTION

A differential investigation has been undertaken to separate physiologically the white pine blister rust (*Cronartium ribicola* Fischer) and the piñon blister rust (*C. occidentale* Hedge., Beth., and Hunt (*7*)[3]), which are morphologically very similar, on species and horticultural varieties of Grossulariaceae. This study was necessary because the uredinial stages of the exceedingly detrimental white pine blister rust and the native piñon or nut pine blister rust, which economically has little importance, are macroscopically indistinguishable, and because both rusts occur in the Pacific Northwest where a control campaign is now being conducted against the recently introduced white pine blister rust, which threatens the highly valuable stands of western white and sugar pines (*Pinus monticola* D. Don. and *P. lambertiana* Dougl.) in the forests of that region. The biometric method recently described by Colley (*4*), by which the uredinial stages of *C. ribicola* and *C. occidentale* in most cases can be separated on the basis of differences in the average lengths and wall thicknesses of the urediniospores, has been shown to be fairly good, but fallible because some specimens are so near the biometric border line separating the two species that their identity became dubious.

To obtain differential hosts, many species and horticultural varieties of foreign and native Grossulariaceae were assembled for propagation. The species of Ribes herein reported include the foreign species and varieties, a group in which the best differential hosts were discovered. Differential results with a group of Ribes from the Pacific Northwest have been reported (*5*).

HOST PLANTS TESTED

The foreign species and varieties of Ribes tested include horticultural varieties of the common red and white garden currant, horticultural varieties of *R. nigrum* L., and miscellaneous species.

The considerable number of varieties of the common garden currant tested in these experiments have been grouped for convenience under

[1] Received for publication Apr. 18, 1929; issued January, 1930.

[2] The writer is under obligation to members of the Office of Forest Pathology for advice and assistance in conducting this investigation; to the late C. S. Sargent, formerly of the Arnold Arboretum, Jamaica Plain, Mass.; to Paul Thayer, of the Ohio Agricultural Experiment Station, Wooster, Ohio; to R. E. Horsey, of Rochester Park, Rochester, N. Y.; to W. H. Alderman, of University Farm, St. Paul, Minn.; to George M. Darrow and Miss M. R. Newman, of the Office of Horticultural Crops and Diseases; to George G. Hedgcock, of the Office of Forest Pathology, for Ribes material for inoculating purposes; to E. P. Meinecke, of the latter office, to J. S. Boyce and the late Ellsworth Bethel, both formerly of that office, and to members of the Office of Blister Rust Control, for culture strains of rusts; and to Carl Hartley, of the Office of Forest Pathology, for valuable assistance in the preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature Cited," p. 120.

the name *Ribes sativum* (*R. vulgare*) until they can be assigned to the proper species. Bunyard (*2*) in his study of this group concluded that certain varieties sprang from the following species: *R. vulgare* Lam., *R. petraeum* Wulf., and *R. rubrum* L. This opinion was also held by Wilmott (*15*) and Thayer (*13, 14*). To quote the latter (*14, p. 316*):

> It so happens that the first of the species [*R. vulgare*] given * * * in which we are interested is historically first so far as introduction is concerned and also first in commercial importance, for there are but few varieties in general cultivation that do not show the influence of this species.

On the other hand, Berger (*1*) and other authorities considered *R. sativum* (Rchbch.) Syme the preferred name for the garden currant. According to Berger, the subgenus representing the group of red currants comprises 15 more or less closely related species, natives of the Northern Hemisphere; *R. sativum* occurs in western Europe and in North America, where it has escaped from cultivation and is subspontaneous from Massachusetts to Ontario and Wisconsin, south to Virginia, and in Oregon and British Columbia. For the most part the varieties studied in these differential tests were obtained from nurserymen; hence certain of the varietal names herein quoted can not be taken as thoroughly dependable because of the possibility that varieties obtained from this source may not always be true to name.[4] Certain varieties are still to be assigned their proper designations. Where names of varieties were uncertain or not found listed, the writer has indicated these facts by quotation marks.

Carefully named varieties of red and white currants were obtained from the following sources:[5] From Paul Thayer: 11 B, London Market; 14 A, Victoria; 28, Holland; 51 B, Wilder; 58 B, White Dutch; 67 B, Transparent. From George M. Darrow: White Versailles, Goliath, and Holland. A variety cited as "Ginkgoides" is a new unnamed variety which originated in the garden of the late Ellsworth Bethel, Denver, Colo. Bethel regarded it as a sport or variety of the common garden red currant.

Horticultural varieties of *Ribes nigrum* were also tested. The black currant, unlike the red, appears to be derived from a single species which has certain variations, as stated by Hatton (*6*). The varieties investigated included Champion, "Carter," Victoria, Black Naples, "Black Dutch," Boskoop Giant, and Blacksmith. The last two varieties were received from Paul Thayer.

Other species tested from the following sources included: From C. S. Sargent: *Ribes succirubrum* Zabel, *R. culverwellii* MacFarl., *R. alpestre* Decaisne, *R. tenue* Jancz., *R. carrierei* Schneider, *R. giraldii* Jancz. From R. E. Horsey: *R. carrierei*, *R. fasciculatum* Sieb. and Zucc., and *R. luridum* Hook and Thom. From G. M. Darrow: The hybrid Van Fleet gooseberry (regarded for convenience as *Grossularia reclinata*). From Block Island, R. I.: *R. alpinum* L.

[4] In attempting to determine the proper names to be applied to each variety investigated by the writer the difficulty just alluded to has been increased by the great confusion that exists in the nomenclature of the common garden currant. The wide distribution of the currant varieties in this country and in Europe, under different names, and the fact that the names sometimes appear, as presented in this paper, in a foreign language, and at other times in the translated form, makes the determination of the correct name exceedingly difficult. There is even further difficulty because of the conflict of opinion among authorities as to priority among the names by which a variety should be known. In general, the names of varieties used in the present investigation are those listed by Thayer (*14*).

[5] Cuttings from a number of varieties were received through the kindness of W. H. Alderman. Unfortunately, these cuttings failed in propagation.

SOURCE OF THE INOCULUM

The inoculum used in the tests was derived from various geographical sources. Uredinial culture strains of *Cronartium ribicola* were obtained from aecia occurring on *Pinus strobus* L. from New England (FP [6] 37073, 38380, 38382, 40300, and 41012), on *P. monticola* from British Columbia (FP 38805), and on *P. monticola* from Scotland (FP 38801). Those of *C. occidentale* were obtained from aecia on *P. edulis* Engelm. from Colorado (FP 36704, 38418, and 41391) and on *P. monophylla* Torrey and Fremont from Nevada (FP 36028, 36922, 38381, and 41365). Uredinial culture strains of *C. occidentale* were also obtained from uredinia occurring on *Ribes aureum* Pursh. from California (FP 36921, 38386) and from Wyoming (FP 38112).

BEHAVIOR OF THE RUSTS IN THE GREENHOUSE

The behavior of the white pine and piñon blister rusts in the uredinial stages in the greenhouse has been described in an earlier paper dealing with the Pacific northwestern Ribes (*5*). The statements made there apply equally well to the present investigation. Leaves of *Ribes sativum* (*R. vulgare*) proved to be susceptible to *Cronartium ribicola* for only a limited period; immature leaves did not become infected, nor did leaves that had commenced to harden. This limited period of receptivity of *R. sativum*, already referred to in the consideration of the closely related wild red currant (*R. triste* Pall.) in the Northwest (*5*), appears to be a very constant physiological character, bearing out the observation of York,[7] who found in his greenhouse tests that plants of *R. triste* from the eastern part of the United States did not develop rust infection from *C. ribicola* until the leaves were two-thirds mature.

On *Ribes nigrum*, *Cronartium occidentale* gave an even more characteristic reaction. Repeated trials with this rust showed that it would infect only scantily, or not at all, very young or middle-aged leaves of *R. nigrum*, whereas it infected moderately and even heavily fully matured leaves at the base of the shoot and produced a large number of telia and uredinia during the fall.

Rust infection in the greenhouse proved much more successful in late summer and fall than at other seasons. This was particularly true for *Cronartium ribicola* on *Ribes sativum*, and for *C. occidentale* on *R. nigrum*. These observations corroborate those of Stakman and Piemeisel (*12*), who reported that *Puccinia graminis* Pers. developed unusually well in late September and early October, a period they found ideal for rust development in the greenhouse; and those of Spaulding (*11*), who found in his investigation with *C. ribicola* that leaves produced from buds developing in late summer or fall readily become infected.

Trouble was experienced with mildew (*Sphaerotheca mors-uvae* (Schw.) Berk. and Curt.) on certain of the species tested, particularly the varieties of *Ribes nigrum*, making it impossible at times to use the plants for the experiments. Even the very susceptible *R. nigrum* would barely become infected with *Cronartium ribicola* when the leaves were attacked by mildew. Fortunately, *R. sativum* variety Fay (Fay's Prolific), with which these experiments were largely

[6] Collection number of specimens for study, Office of Forest Pathology, Bureau of Plant Industry.

[7] YORK, H. H. FIELD STUDIES OF CRONARTIUM RIBICOLA IN THE WHITE MOUNTAINS OF NEW HAMPSHIRE. [Unpublished manuscript.]

concerned, was not affected with mildew. This freedom from mildew shown by the Fay variety was reported by Salmon and Wormald (*9*) in England; they observed that a severe outbreak of the American gooseberry fungus occurred on Raby Castle, while the Fay variety in the immediate neighborhood remained uninfected.

So far as the writer observed, there was no apparent difference in the length of time required for the first signs of rust infection to appear on susceptible leaves and on resistant ones of the same species or variety. Fertile pustules matured in a shorter period on susceptible leaves than on resistant ones; aborted or abnormal pustules developed after a somewhat longer period of time. Peltier (*8*) cited a somewhat similar condition as the result of his infection studies with stem rust of wheat.

METHODS

The methods followed in obtaining the results set forth in this paper have already been described (*5*). As previously stated, these methods were based upon those used by cereal-rust investigators but adapted to the requirements of the Ribes host.

PRODUCTION OF INOCULABLE LEAVES BY MEANS OF COLD STORAGE

Since the differential study involved in the main an extensive experimentation with varieties of *Ribes sativum* (*R. vulgare*), it was necessary to have plants with inoculable leaves for all times of the year, particularly in the fall. Normally, *R. sativum* produces a single crop of leaves in the spring, which by fruiting time become fully matured and hardened, thereby being rendered unfit for inoculation purposes. To insure leaves for continuous inoculation purposes, a cold-storage method was used. Plants of *R. sativum* which had passed through their active growing period or were in a dormant condition were sunk in damp sphagnum moss in flats and placed in a cold-storage room where the temperature was kept approximately at the freezing point. Such plants required watering about every three weeks to prevent their drying out. Plants thus kept at low temperature for two or preferably three months break into leaf readily upon withdrawal from the storage room. They should at first be kept in a cool room after repotting in fresh soil and gradually be taken to warmer sections of the greenhouse. Plants so treated will produce leaves within a month or longer, the length of time depending upon the season of the year. Plants placed in storage during late spring or early summer can thus be made available for fall work, and plants kept over winter can be utilized during the early spring months. Inasmuch as field scouting in the Pacific Northwest, where the piñon and white pine blister rusts are to be found, is carried on mainly during the late summer and fall, this procedure for insuring inoculable leaves at this time is an essential part of any method of identifying by inoculation tests the Cronartium specimens which the scouts find on Ribes.

RECORDING DATA

The inoculated plants were classified by infection types, based on the pathologic symptoms produced by the rusts on Ribes leaves. The symbols indicating these inf tion types and the relative quan-

tity or abundance of uredinia produced on the infected leaves have been fully described in a paper recently published (*5*). The symbols indicating the types of infection are described briefly as follows:

Resistant types:

Immune—

○ No uredinia formed; hypersensitive or necrotic areas present or lacking.

Resistant—

◑ Uredinia minute; associated with hypersensitive or necrotic areas.

Susceptible type:

● Uredinia normal size; no hypersensitive or necrotic areas.

The relative quantity or abundance of uredinia produced on the infected leaves was also expressed in the note taking by symbols. They are described briefly as follows:

=, *Trace.*—Uredinia bare trace, or very few in number.
−, *Slight.*—Number of uredinia below normal, scanty.
±, *Moderate.*—Medium production of uredinia; normal infection.
+, *Heavy.*—Infection heavier than medium.
++, *Very heavy.*—Extremely abundant production of uredinia.

In recording the abundance of uredinia produced on each host species or variety tested, a rating was given the plant as a whole.

CORRELATING DATA

The symbols indicating the abundance of urediniospores on uredinia-bearing leaves of a given Ribes species or variety were reduced to a numerical basis and averaged (*5, p. 672*). Table 1 gives the numerical expression for each symbol.

TABLE 1.—*Mathematical as related to symbolic expression of the abundance of uredinia production*

Symbols for relative abundance of uredinia produced on infected leaves	Equivalent	Range of class [a]	Mid value of class [a]	Abundance of uredinia
		Per cent	*Per cent*	
=	(×)	Less than 5	2.5	Trace.
−	×[b]	5–35	20.0	Slight.
±	××[b]	35–65	50.0	Moderate.
+	×××[b]	65–85	75.0	Heavy.
++		85–100	92.5	Very heavy.

[a] The percentage values are rational expressions of the abundance of uredinia production based on the maximum uredinia development on completely infected leaves of *Ribes nigrum*, under favorable conditions, which were taken as a standard. In converting the symbols into numbers, each was assigned the mid value of the class that it represents.

[b] Symbols used in previous white pine blister rust investigations. See Spaulding (*11*), Range percentage and mid values of classes do not apply to these symbols.

In obtaining the averages shown in Figures 1 and 2, the rating for each plant tested was weighted by the number of spore-producing leaves on the plant.

PHYSIOLOGICAL COMPARISON OF CRONARTIUM RIBICOLA AND C. OCCIDENTALE

ON MISCELLANEOUS FOREIGN RIBES SPECIES

In the differential study of the white pine and piñon blister rusts on foreign species and horticultural varieties, a number of miscellaneous foreign species were tested. The study also included 62 plants (367 leaves) of *Ribes nigrum* inoculated with *Cronartium ribicola* and 72 plants (466 leaves) inoculated with *C. occidentale*,

together with 250 plants (1,713 leaves) of the group *R. sativum* (*R. vulgare*) inoculated with the first-named rust, and 344 plants

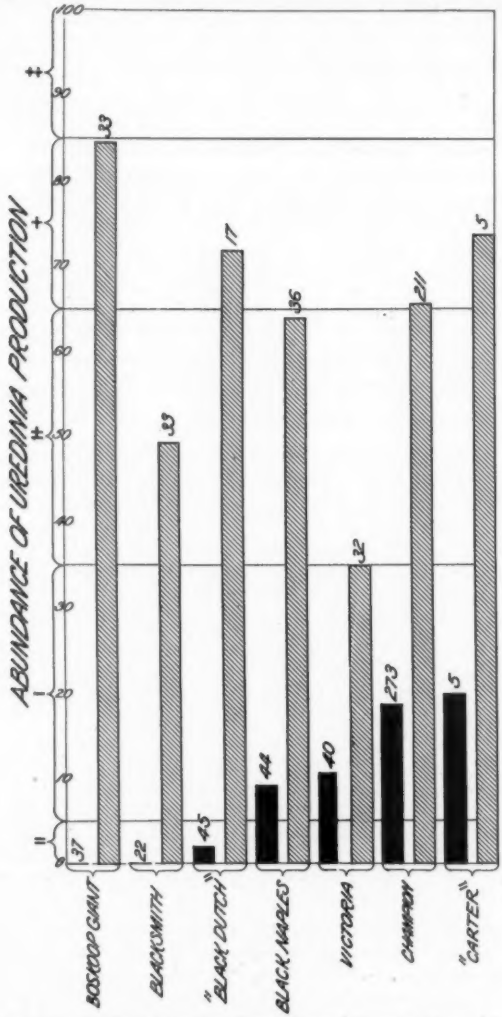

FIGURE 1.—Results of inoculations with *Cronartium occidentale* and *C. ribicola* on horticultural varieties of *Ribes nigrum*. The black bar shows the average abundance of fertile uredinia of *C. occidentale* for all the leaves inoculated. In averaging, the results on the different plants were weighted according to the number of spore-producing leaves. The figures above the bars show the total number of leaves inoculated. The symbols at the left margin are explained on page 109. The figures at the left margin are on a scale on which 100 is the maximum uredinia production for *C. ribicola* on fully susceptible leaves of *R. nigrum*. The shaded bar shows the same for leaves inoculated with *C. ribicola*

(2,364 leaves) inoculated with the latter. The results are given in Figures 1, 2, and 3 and in the discussion following.

Among the miscellaneous foreign Ribes species tested with *Cronartium ribicola* and *C. occidentale*, negative results were obtained for both rusts on each of the following: *Ribes luridum* (3 plants, *C. ribicola*; 3 plants, *C. occidentale*); *R. alpestre* (2 plants, *C. ribicola*; 1 plant, *C. occidentale*); *R. diacantha* (1 plant, *C. ribicola*; 1 plant, *C. occidentale*); *R. alpinum* (11 plants, 109 leaves, *C. ribicola*; 8 plants, 55 leaves, *C. occidentale*); *R. culverwellii* (5 plants, *C. ribicola*; 6 plants, *C. occidentale*); *R. giraldii* (9 plants, *C. ribicola*; 3 plants, *C.*

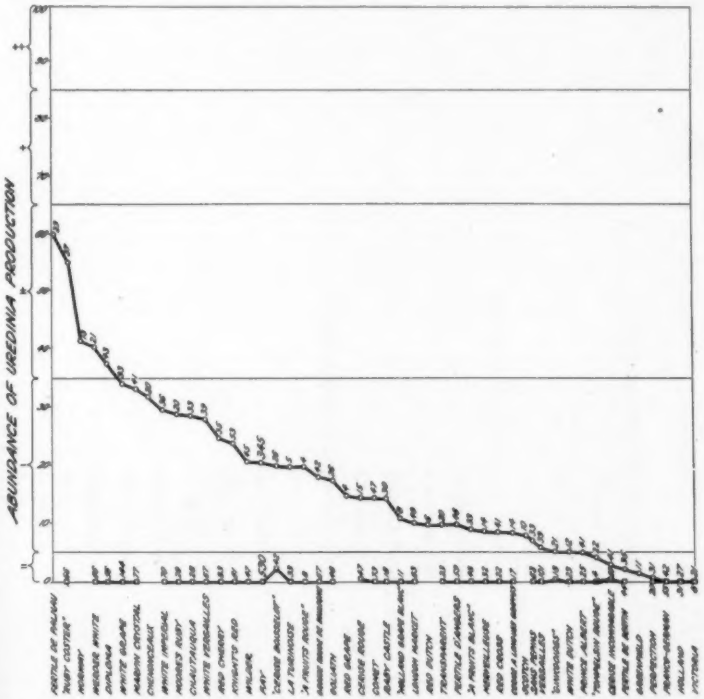

FIGURE 2.—Results of inoculations with *Cronartium ribicola* and *C. occidentale* on horticultural varieties, grouped under the name *Ribes sativum* (*R. vulgare*), stated in terms of the average abundance of uredinia production. In averaging, the results on the different plants were weighted according to the number of spore-producing leaves. The figures above the points on the graph show the total number of leaves inoculated. The symbols at the left margin are explained on page 109. The figures at the left margin are on a scale on which 100 is taken as the maximum uredinial production for *C. ribicola* on fully susceptible leaves of *R. nigrum*. Hollow circle indicates *C. ribicola;* hollow square, *C. occidentale*

occidentale). Clinton and McCormick (*3*) reported negative results with *C. occidentale* on *R. alpestre*, *R. alpinum* ♀, *R. giraldii*, and *R. luridum*. They also gave negative results with *C. ribicola* on all these hosts except *R. luridum* which averaged fair minus (F−) in 15 tests, and *R. alpinum* ♂, fair minus (F−), in 11 tests. Spaulding (*11*) reported negative results for this latter host species. *R. alpinum* in Europe was said by Schellenberg (*10*) to develop 70 to 90 per cent of infection with *C. ribicola*. These findings do not agree with the results of investigators in this country working with

R. alpinum. Apparently within the species *R. alpinum* there are strains which differ in susceptibility.

Both Cronartiums produced a heavy amount of normal uredinia on *Ribes carrierei* and *R. succirubrum*. A moderate infection of the same type with both rust species was obtained on the hybrid Van Fleet, and a very heavy infection on the Poorman gooseberry varieties, respectively. Spaulding (*11*) cited Poorman as a moderate host for *C. ribicola;* Clinton and McCormick (*3*) reported poor

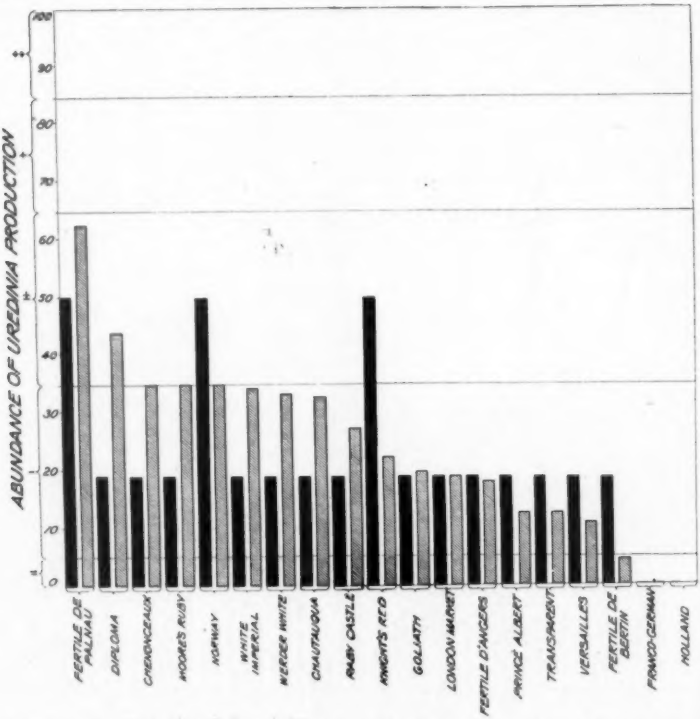

FIGURE 3.—Comparison of results of greenhouse inoculations with *Cronartium ribicola* on horticultural varieties of the group *Ribes sativum* (*R. vulgare*) obtained by the writer and those obtained by Spaulding and his associates. In averaging the writer's results for each variety compared, each plant inoculated was given a mathematical rating equivalent to the mid-point of the degree of infection class to which each belonged. The symbols at the left margin and their conversion to an arithmetical basis are explained on page 116. The shaded bar shows the average abundance of uredinia for the total number of plants inoculated by the writer. The black bar shows the average abundance of uredinia for the total number of inoculated plants reported by Spaulding. The conversion of infection ratings to a basis comparable with the writer's ratings is explained on p. 116

results on a large gooseberry and Smith (gooseberry) for both Cronartiums.

Results on *Ribes fasciculatum* (5 plants, 44 leaves) and *R. tenue* (3 plants, 52 leaves) were negative for *Cronartium occidentale*. On the former host (5 plants, 34 leaves) and on the latter (6 plants, 72 leaves) *C. ribicola* produced a slight (−) infection. The average abundance of uredinia production for these 72 leaves of *R. tenue* gave a numerical value (based on the scale of 100 for completely infected leaves of *Ribes nigrum*) of 6.5. Clinton and McCormick (*3*)

reported poor plus (P+) results for *R. tenue* with *C. ribicola* in 10 tests, and Spaulding (*11*) obtained heavy infection on this host in two tests. Possibly there are also in this instance within the given Ribes species strains which differ with regard to rust susceptibility.

ON HORTICULTURAL VARIETIES OF RIBES NIGRUM

In Figure 1 are given the results of a physiological comparison of *Cronartium ribicola* and *C. occidentale* on horticultural varieties of *Ribes nigrum*, a host which is extremely susceptible to *C. ribicola* and which remains highly susceptible throughout the growing season. The European black currant can be regarded as a nurse plant for white pine blister rust. So serious a danger is it to the production of white pine timber that it is now regarded as a definite menace to the white pine timber supply of this country.

Ribes nigrum varieties, Blacksmith and Boskoop Giant, failed to become infected with *Cronartium occidentale*. Infection (type ●) on the other varieties tested with piñon rust was secured on the older fully matured leaves at the base of the shoot. Among the varieties extensively tested, Champion proved to be the most susceptible to *C. occidentale*, but only during the fall period; 73 (27 per cent) susceptible leaves of the 273 tested leaves of this variety produced a moderate amount (±) of uredinia. The average uredinia production calculated for these 73 leaves alone was 64.6 (based on the scale of 100 for completely infected leaves of *R. nigrum*). This numerical value was somewhat lower than the value calculated for the amount of uredinia (+) produced by *C. ribicola*, 76.3, for 182 (86 per cent) susceptible leaves out of 211 leaves tested for the same host. Clinton and McCormick (*3*) obtained only poor results for inoculation of *C. occidentale* on *R. nigrum* and *R. nigrum aconitifolium;* the uredinial stage from *R. gracillimum* on *R. nigrum* failed. Hedgcock, Bethel, and Hunt (*7*) list *R. nigrum* as one of the poorest hosts for *C. occidentale* in their inoculation tests.

ON HORTICULTURAL VARIETIES OF RIBES SATIVUM (R. VULGARE)

In Figure 2 is shown the comparison between *Cronartium ribicola* and *C. occidentale* with respect to the amount of uredinia produced on varieties of *Ribes sativum* (*R. vulgare*) for the total number of leaves inoculated. With the exception of four varieties—Cerise Incomparable, Cerise Rouge, "Cerise Boisselot," and "Ginkgoides"—on all of which *C. occidentale* produced infections of the resistant type, the other tested varieties of *R. sativum* were apparently quite immune (type ○) to *C. occidentale*. Positive results on *R. vulgare* (*R. sativum*) with *C. ribicola* have been published by Spaulding (*11*) and by Clinton and McCormick (*3*). In written communications to the writer, Bethel reported that he had found the "Ginkgoides" variety to be only slightly susceptible to *C. occidentale* under field conditions, and N. Rex Hunt stated that on September 15, 1918, he observed, at Bayfield, Colo., *R. aureum* heavily infected with *C. occidentale*, associated with a red currant almost every leaf of which was infected with the same rust. Hunt did not report the varietal name of this currant or the degree of infection of the rust. He also observed cultivated gooseberry and *Grossularia inermis* (Rydb.) C. and B. infected with *C. occidentale*. Clinton and McCormick (*3*), as a result of their rust-infection studies of leaves in Petri dishes, and of pot

inoculations, reported negative results for infection with *C. occidentale* on *R. sativum* variety Fay, small currant, and white currant.

The largest number of tests in the present study were made on *Ribes sativum* variety Fay with culture strains of *Cronartium ribicola* from New England and British Columbia on 61 plants (345 leaves). The following culture strains of *C. occidentale* were tested on 100 plants (530 leaves) of the same host variety: One strain from Colorado, on 25 plants (130 leaves); two strains from California (Monrovia), on 29 plants (143 leaves); and three strains from Nevada (Minden), on 46 plants (257 leaves). All the horticultural varieties of *R. sativum* producing fertile uredinia of *C. ribicola* could be classified as belonging to the susceptible type ●. (See p. 110.) Of the 48 varieties tested with *C. ribicola*, 36 produced normal uredinia; 9 produced only resistant-type uredinia; 3 remained immune. The 9 varieties which were resistant (type ◑) under the conditions of the inoculation experiments were: "A Fruits Rouge," "Tompelsin Rouge," "Ginkgoides," Fertile de Bertin, London Market, Perfection, Red Grape, La Turinoise, and White Dutch.

So far as tested, the three varieties Franco-German, Holland, and Victoria were immune (type ○) to both rusts. Further experimentation may duplicate the results already obtained with these three varieties, which so far have proved immune to both Cronartiums, and eventually they may be conclusively regarded as valuable red currants for planting in localities where the growing of this Ribes is desired along with that of white pines. Spaulding (*11*) listed varieties of red currant tested under the following varietal names as resistant to *C. ribicola* but not entirely immune: Eyatt Nova, Franco-German, Holland, London (London Market), Rivers (Rivers Late Red), and Simcoe King. The stock of Franco-German and Holland tested by the writer and found to be immune did not come from the same source as that used by Spaulding.

The three currant varieties listed above by the writer as being immune to *Cronartium ribicola* under the conditions of the foregoing inoculation experiments belong to two of the large groups into which the cultivated currants have been classified (*1, 2, 14*). The Victoria variety belongs to the group "*Ribes rubrum* and hybrids" and was reported by Thayer (*14, p. 386*) as being resistant to disease. In this group is also found the London Market (London) variety, which both Spaulding (*11*) and the writer found to be resistant but not immune.

In the group "*Ribes petraeum* and hybrids" are found the Holland (Long Bunch Holland) and Franco-German varieties, the latter regarded by Thayer (*14, p. 391*) as synonymous with Holland. Concerning the Holland variety, the chief value of which is in its extreme lateness and great resistance to heat and drought, Thayer stated that in the prairie region this variety had done very well.

Prince Albert (Rivers Late Red), which Spaulding (*11*) found to be resistant but not immune to *Cronartium ribicola*, is also in the *Ribes petraeum* group. The same investigator reported (*11, p. 19*) a moderate degree of infection on *R. petraeum*. Until further information is available, it is not wise to favor the cultivation of the Prince Albert (Rivers Late Red) variety, as Thayer suggests (*14, p. 394*), on account of the seeming resistance of *R. petraeum* to the blister rust, for both Spaulding (*11*) and the writer have found that

variety to be susceptible (type ●), although only a slight infection (−) was secured. Further experimentation will be necessary, however, before a final, definite opinion concerning the disease resistance of this variety can be obtained.

During a visit to Norway the writer had the opportunity to observe a variety of red currant known as "Red Dutch," which was highly resistant to white pine blister rust. This variety was observed in August, 1927, in the nursery of the Agricultural College at Ås, Norway, growing in immediate proximity to the White Dutch variety, which was heavily infected with the rust. Conditions for rust infection were particularly favorable during 1927 in Norway, and a heavy production of fruiting bodies of species of rusts generally was observed at that time. An examination of plants of the Red Dutch variety did not reveal the presence of sori on any of the leaves. Had the variety been susceptible at all, it is reasonable to believe that it would have become infected during 1927.

Mr. Ivar Jørstad, State mycologist of Norway, Botanisk Museum, Oslo, and Professor Doctor Hagem, of the Botanisk Museum, Bergen, Norway, both regard the Norwegian Red Dutch variety as highly resistant to the white pine blister rust. Jørstad, in correspondence with the writer, has given the following information concerning the synonymy of this variety:

> I can inform you concerning the synonymy of the red-currant strains in question. I have conferred with Mr. P. Stedje, leader of the Pomological Experiment Station at Hermansverk in Sogn. He is our best specialist in this matter and has even quite a few American red-currant strains in culture (these he has obtained from Paul Thayer in Ohio).
>
> Mr. Stedje tells me that there is a great confusion concerning the names of one and the same strain. The Norwegian "Rød hollandsk druerips" (i. e., Red Dutch grape currant), which is the one resistant to *Cronartium ribicola*, is not identical with the Danish "Rød hollandsk druerips," but with their "Rød spansk" (i. e., Red Spanish). Our "Rød hollandsk druerips" is further not identical with the American "Red Dutch," neither with Long Bunch Holland nor Victoria, but it is very similar to Prince Albert and to Rivers Late Red (the two latter are possibly identical). Our "White Dutch," which is susceptible to the rust, is, according to Mr. Stedje, not closely related to our "Red Dutch," and the same is the case with the American "Red Dutch." Although the two latter possess the same name, they are entirely different.

From observations and inquiry made in Norway, the writer is inclined to regard the Norwegian Red Dutch variety of red currant as having great possibilities as a horticultural variety, particularly for planting in white pine areas of the United States when the growing of Ribes is desired along with that of white pine. Comprehensive artificial-inoculation experiments will be necessary, however, to demonstrate completely the apparent immunity of this particular variety under cultural conditions in the United States, before it can be recommended for use in this country.[8]

Figure 3 shows a comparison between the greenhouse inoculation results published by Spaulding (*11*) for certain varieties grouped under the name *Ribes vulgare* (*R. sativum*) and those obtained by the writer on the varieties of the same group, of which more than a single plant was tested. To make the results reported by Spaulding

[8] Preliminary inoculation experiments performed in 1929 at the Royal Botanic Garden, Edinburgh, Scotland, in which plants of the Norwegian Red Dutch currant were inoculated with a Scottish strain of *Cronartium ribicola* under rigorously controlled conditions demonstrated the Norwegian variety to be immune to white pine blister rust. See HAHN, G. G. PRELIMINARY REPORT ON A VARIETY OF RED CURRANT RESISTANT TO WEYMOUTH PINE RUST. Trans. Bot. Soc. Edin. 30: 137–146, illus. 1929.

and his associates comparable with those of the writer, it seeemd best to reduce the infection rating given by them to a numerical basis, on a scale in which 100 represents the abundance of uredinia on completely infected plants of *R. nigrum* with *Cronartium ribicola* under favorable conditions. The symbol of a cross within parentheses (×) was interpreted to indicate a degree of infection more than O but less than 5 per cent (trace of infection); × for 5 to 33 per cent (slight infection); ×× for 33 to 67 per cent (medium infection); and ××× for 67 to 100 per cent (heavy infection). This interpretation was approved by Spaulding. The midpoints of the classes are therefore as follows: (×), 2.5; ×, 19; ××, 50; and ×××, 83. This method of reducing ratings to a numerical basis is practically the same as that used by the writer in averaging his own data (see p. 109), and the converted results of the two investigations conducted under greenhouse conditions show a reasonable agreement.

Ribes sativum can be considered only a fair host for *Cronartium ribicola*, for it produces fewer uredinia than most Ribes species even when infected under the most favorable conditions. Clinton and McCormick (*3*), in averaging their results for infection of *C. ribicola* on *R. vulgare* (*R. sativum*) in Petri dishes and with pot inoculations, reported the following: On *R. vulgare*, F− in 22 tests; on Fay, F− in 24 tests; on *R. vulgare* (small), F− in 18 tests; on *R. vulgare* (white), F in 15 tests. The symbols used by Clinton and McCormick are as follows: O, failure; P, poor; F, fair; G, good; and E, excellent. To quote these writers:

> As a rule poor indicates that fewer than five sori developed. Excellent implies the development of 40 or more on a leaf or leaves in a Petri dish and an even greater total number on the leaves of a plant in a pot. Good and fair are intermediate terms.

So far as a comparison between the Petri-dish method of inoculation of *R. sativum* (*R. vulgare*) and the pot inoculations was concerned, somewhat better results were obtained with the latter method.

IMPORTANCE OF RIBES SATIVUM (R. VULGARE) AS A DIFFERENTIAL HOST

A consideration of the foregoing results indicates that among the horticultural varieties of the group *Ribes sativum* (*R. vulgare*), certain varieties tested upon a comparative basis—Chautauqua, Comet, Diploma, Fay, Fertile d'Angers, Goliath, Grosse Rouge de Boulogne, Knight's Red, Marvin Crystal, Raby Castle, Red Cherry, Red Cross, "Ruby Coster," Moore's Ruby, Werder White, White Grape, White Imperial, White Versailles, and Wilder—have shown themselves to be good differential hosts. The most extensive comparative tests were made with the variety Fay (Fay's Prolific). Repeated tests with Fay have shown it to be a most dependable separating host, provided due attention is given to the securing of suitable leaves for inoculation purposes; the variety lends itself readily to propagation both in and out of the greenhouse. Its freedom from mildew and its adaptability to the cold-storage methods above described make it indispensable as a differential host.

Differences obtained on other host species were not definite enough to be relied upon. The attempts to infect *Ribes nigrum*, varieties Blacksmith and Boskoop Giant, with *Cronartium occidentale* should be carried farther before the immunity of these varieties herein reported can be unqualifiedly accepted. The readiness with which

C. ribicola infects species of Grossulariaceae generally has made it impossible so far to find a species which is resistant or immune to *C. ribicola* and not to *C. occidentale*. Whenever Ribes species have shown resistance to *C. ribicola*, they have manifested the same reaction to the piñon rust. Where Ribes have shown a difference in reaction between the two, this difference has consisted in better production of uredinia by *C. ribicola* than by *C. occidentale*.

METHOD OF DIAGNOSING AN UNKNOWN CRONARTIUM ON RIBES

It has already been demonstrated (*5*) that the native Pacific northwestern Ribes in areas where the two Cronartiums are expected to intermingle are generally susceptible to both rusts. Any Cronartium found on Ribes in Idaho and the adjoining region is therefore an unknown. Upon receipt of such a specimen from the field, slides of the spores of the unknown Cronartium are made for measurement and the remainder straightway inoculated upon *Ribes sativum* (*R. vulgare*) variety Fay. *R. aureum*, a congenial host, is inoculated at the same time. Such an inoculation determines the viability of the unknown spores which are to be tested and also is the means of securing a vigorous stock culture for any further investigation which may be necessary. As an added check, duplicate plants of the Fay variety in the same condition of leaf are also inoculated, as a parallel test, with a known strain of *C. ribicola*. This check on the test of the unknown shows whether the leaves of the variety of *R. sativum* are in the right condition for inoculation; a precaution such as has been pointed out is highly desirable with this host. Results should be obtained within 14 days, the length of time necessary for fertile pustules to appear. The interpretation of the results is explained in Table 2.

TABLE 2.—*Method of interpreting results of inoculation tests with an unknown Cronartium from Ribes*

[Positive denotes the production of uredinia (type ●) on some of the inoculated leaves; negative denotes absence of uredinia]

Result of—			Interpretation
Inoculations with unknown Cronartium		Parallel inoculations with *C. ribicola* on Ribes sativum (vulgare) variety Fay	
On Ribes sativum (vulgare) variety Fay	On Ribes aureum		
Positive	Positive	Positive	The unknown is C. ribicola.
Negative	do	do	The unknown is C. occidentale.
Do	do	Negative	The leaves of the variety Fay were not in a receptive condition; the test must be repeated on better host material.
Do	Negative	Positive	The spores of the unknown are not viable; no determination possible.

For decisive negative results, it is of course desirable to use several plants in each category, or to repeat the entire test a few days later. If, for example, the infection with *Cronartium ribicola* on Fay is not very abundant, a failure of the unknown on a single plant of the same host might easily be the result of a slight difference in condition of susceptibility between the different plants of Fay; or it might mean that the spores of the unknown were in a less vigorous condition than

those of the known *C. ribicola;* in either case, if there were no replication of the plants in the experiment, obviously it would be unsafe to attempt to name the unknown.

LIMITATIONS OF THE METHODS OF DISTINGUISHING THE TWO RUSTS

The diagnosis of an unknown Cronartium upon a physiological basis under the conditions worked with by the writer is a somewhat slow process when immediate results are required. Good spore material can be identified by the inoculation method in approximately two weeks; but if the material is poor and must first be cultured on a congenial host, e. g., *Ribes aureum*, *R. odoratum* Wendl., or *R. gracillimum* C. and B., to secure a supply of spores for the differential test, an additional two weeks' delay results. The number of conditioning factors also complicate the procedure of diagnosis. As already stated, inoculable leaves must be available and the proper environmental conditions of temperature, humidity, and light supplied. Ordinarily the high temperatures of summer, particularly in the greenhouses, make it difficult to get satisfactory results, unless such temperatures are controlled.

It must also be kept in mind that in the experiments herein reported only a comparatively few culture strains, representing a limited number of geographical sources, of each species were tested. There is the possibility that certain strains of *Cronartium ribicola* might be found which would not infect *Ribes sativum* (*R. vulgare*) var. Fay, the variety investigated most extensively. There is also, of course, the possibility that a strain of *C. occidentale* might be found which would infect Fay.

Other methods of differentiating the two Cronartiums have been studied. For tentative diagnosis the quicker but less certain biometric differential method of Colley (*4*), discussed in an earlier part of the paper, can be used and supplemented with the physiological test.

It may be noted that neither the physiological nor the biometric method which has been developed for distinguishing the two rusts is of much use for unknown specimens collected late in the season in the Pacific Northwest. Such material commonly shows teliospores only. Both the methods above referred to require the presence of urediniospores. The need of the blister-rust control workers for a method which will enable them to recognize *Cronartium ribicola* whenever and wherever they find it will not be fully met till a method is found of distinguishing between it and *C. occidentale* in the telial stage. G. G. Hedgcock, as a result of long experience with the *C. occidentale*, is able in most cases to distinguish readily by inspection this fungus from *C. ribicola* in the telial stage, both by the color (*7*) and by the number and vigor of the telial columns. Of 32 specimens of the former and 12 of the latter species, on Ribes hosts common to both, he correctly identified all but 2 without seeing the labels. The writer was able to identify correctly by inspection 37 of these 44 specimens. The macroscopic differences between the two rusts in the telial stage are so slight, particularly in weathered specimens, and so difficult to describe, that they are inadequate for general diagnostic use. An effort is now being made in the Office of Forest Pathology to develop a microscopic method of distinguishing between the telia or sporidia of the two species, but no constant difference has thus far been found.

SUMMARY

Foreign Ribes species and horticultural varieties were inoculated with a number of strains each of *Cronartium ribicola* and *C. occidentale* in the greenhouse at Washington, D. C., to discover physiological differences between the white pine and piñon blister rusts, which morphologically are very similar in their uredinial and telial stages. In the Pacific Northwest, where a control campaign is now being conducted against the recently introduced serious and menacing white pine rust, workers are faced with the perplexing problem of distinguishing macroscopically this detrimental rust on Ribes from the native piñon rust which economically has little importance.

Essential physiological differences were established between the two Cronartiums in the uredinial stage under artificial greenhouse conditions. A large number of horticultural varieties of the common garden currant, grouped for convenience under the name *Ribes sativum* (*R. vulgare*) were found to be immune to *C. occidentale* and susceptible to *C. ribicola*. Extensive and thorough tests with the Fay variety (Fay's Prolific) have demonstrated this variety to be particularly adaptable for experimental use as a dependable differential host. More limited comparative tests with other currant varieties indicated similar relations. Of 48 varieties inoculated with *C. ribicola*, all except 3 became infected; of 41 varieties inoculated with *C. occidentale* only 4 became infected, and these to a negligible degree, producing only uredinia of the resistant type.

The three varieties of the group *Ribes sativum* (*R. vulgare*)—Franco-German, Holland, and Victoria—which proved to be immune, so far as tested, to both rusts, may eventually after further examination prove to be valuable red currants for planting in localities where the growing of this Ribes is desired along with that of white pines. Further rigorous inoculation experiments will be necessary, however, to demonstrate completely the apparent immunity of these three varieties.

Ribes nigrum, the most receptive of all uredinial hosts to *Cronartium ribicola*, developed only scant infection with *C. occidentale* in the total number of tests made. *C. occidentale* further differed from *C. ribicola* in its reaction on this host, in that it infected fully matured leaves only during the fall period much more readily than it did younger leaves, which were fully susceptible to *C. ribicola*. Of the varieties of *R. nigrum* extensively tested, Champion was most susceptible to *C. occidentale*. In the limited number of tests made the Blacksmith and Boskoop Giant varieties were immune to *C. occidentale*.

Tests with 12 miscellaneous foreign Ribes species showed only two *R. tenue* and *R. fasciculatum*, which were susceptible to *Cronartium ribicola* and immune to *C. occidentale*. These two hosts should be further tested to determine the constancy of the very limited differential results obtained.

The procedure for diagnosing unknown uredinial material on Ribes from the Pacific Northwest by the greenhouse method is described. The physiological method of differentiating the white pine and piñon blister rusts, performed in accordance with the conditions under which the writer worked, while somewhat slow, appears much more certain than any other method now available of distinguishing *C. ribicola* and *C. occidentale* in the uredinial stages.

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ALFALFA ROOT INJURIES RESULTING FROM FREEZING[1]

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INTRODUCTION

It is generally recognized that winter killing is one of the most common causes of the loss of stands of alfalfa throughout the northern alfalfa-growing sections of North America. Much has been written in recent years on the value of planting only hardy strains of alfalfa as well as on the relation of frequency of cutting and other cultural conditions to the longevity of the stand and the correlation between certain physiological and chemical factors and hardiness. It is not the purpose of this paper, however, to review the extensive literature dealing with winter killing, but rather to record the results of experiments that have a bearing on one phase of this problem.

Not until recently has the attention of investigators been directed to a study of the individual plants that have been injured by freezing. Heretofore it has been customary to consider the stand as a whole; consequently, it has been difficult, if not impossible, to explain the cause of certain types of lesions found on alfalfa roots.

The writer recently described and illustrated (*6*)[3] diseases of alfalfa designated as heart rot and collar rot, which were considered as being directly or indirectly caused by environmental conditions during the winter months. Since then Jones (*2*) has published a paper in which he discussed from a histological viewpoint the origin of such lesions. Steinmetz (*4*) illustrated an alfalfa plant showing heart rot due to artificial freezing. Russell and Morrison (*3, p. 35*) have published pictures of alfalfa plants showing the effects of winter injury. Throckmorton and Salmon (*5*) showed two plants with lesions just below the crown which they attributed to winter injury. Melchers[4] also has described a condition that he thought might be due to freezing injury.

Although, with the exception of Steinmetz, the above-mentioned writers attributed the lesions described to winter injury, they did not record having reproduced them. It is the purpose of the present paper to give the results of experiments which were designed to determine the origin of the different types of lesions attributed to winter injury and to learn something regarding the conditions necessary to reproduce them.

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[2] The writer is indebted to Dr. F. R. Jones for assistance in freezing the plants at Madison, Wis., and in making and interpreting some of the slides used in the histological phase of this work; to Dr. L. R. Jones for the use of the freezing equipment at Madison; and to Prof. S. C. Salmon, of the department of agronomy at the Kansas State Agricultural College, for the use of his freezing chamber.

[3] Reference is made by number (italic) to "Literature Cited," p. 142.

[4] MELCHERS, L. E. CROWN AND ROOT ROT OF ALFALFA (UNDET.). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 9: 54. 1925. [Mimeographed.]

METHODS

For the most part the plants used in these experiments were of either the Kansas common or the Grimm variety from 6 months to 1 year old. Some of the plants were grown in the greenhouse while others were taken from the field and transplanted to pots in the greenhouse either just before freezing or a month or more prior to their use. In some experiments the plants were frozen in the soil in pots of different sizes, and in others they were removed from the soil before being frozen. In a few instances the plants were frozen just as they stood in the soil in the greenhouse or in the field. In the case of plants removed from the soil preparatory to freezing, they were planted again as soon as they came from the freezing chamber either in soil in pots or in beds in the greenhouse.

Several different types of freezing apparatus have been used. In a number of experiments the freezing chamber belonging to the agronomy department of the Kansas State Agricultural College and directly under the supervision of S. C. Salmon was used. This equipment has been described by Hill and Salmon (*1*). In other cases the freezing was accomplished by inserting the plants freed from soil in a sheet-iron chamber about 4 by 10 by 16 inches in size, and which was cooled by means of salt and ice. Plants were frozen also by placing over them as they stood in the soil a can containing 100 pounds of ice and various amounts of salt. This can had a raised bottom into which the plants fitted in such a way that they were surrounded by the salt and ice mixture above and on the sides; the bottom of the can rested on newspapers on the ground. In some experiments conducted at Madison, Wis., plants were frozen by placing them in soil in specially designed pots which fitted into artificial ice cans resting in the brine of a refrigerating machine. The temperature was recorded either by thermometers placed near or among the plants as seemed most expedient or with thermographs or thermocouples. Where thermometers were used a record of the temperature was made every few minutes, so that the temperature to which the plants were exposed was known fairly accurately at all times. Plants of each lot were held unfrozen as controls for comparison with those frozen.

Certain other details of methods will be given where pertinent in connection with a discussion of the experimental data.

EXPERIMENTAL DATA

PRODUCTION OF PHLOEM INJURY AND HEART ROT BY FREEZING ALFALFA ROOTS

Several thousand plants have been frozen during the two years that these experiments have been in progress. The plants used differed in the degree of hardening, and the methods were sufficiently diverse so that varying degrees of injury were inflicted. Relatively few winter-injury lesions of the type under consideration developed even among those plants that were more or less severely damaged as indicated by the death of the stems and buds. Only those experiments in which one or more plants developed such lesions will be recorded here. For convenience in discussion the terms "phloem injury" and "heart rot" are used to designate the injury to that part of the root lying outside of the cambium and to that part inclosed by the cambium, respectively. Although these injuries have the

same origin, they do not always appear in the same root; hence it is convenient to have some name by which one or the other may be designated. This usage is consistent with that adopted by Jones (*2*). The term "phloem injury" as here used is synonymous with collar rot as used in a former paper (*6*). The frozen plants were examined frequently, some of them being removed from the soil after various intervals of time, while others were allowed to grow for several months before they were finally examined. As was pointed out in a previous publication (*6, p. 6*), not all of the decay of the center of the crowns and upper part of the taproots of alfalfa plants is due to freezing injury. Plants with more or less hollow crowns and taproots can be found in regions where severe freezing never occurs.

In Table 1 are listed the types of injury produced by freezing 8 to 10 months old Kansas common alfalfa plants which had been growing all summer in the field but which were brought into the greenhouse on December 1, 1926, and set in 8-inch pots, where they grew at temperatures ranging from 60° to 90° F. As they were not used in freezing tests until January and February, they were nonhardened. In no case did any plants that were not subjected to experimental freezing develop symptoms of injury similar to those under consideration. The pots of plants were placed in the freezing chamber belonging to the agronomy department, where they were exposed to various temperatures for different periods of time. After being frozen the plants were held in the greenhouse for several months before the final observations were made. As the temperature of the freezing chamber varied considerably at times, the range as well as the average temperature is given.

TABLE 1.—*Phloem injury and heart rot produced as a result of freezing nonhardened Kansas common alfalfa plants in soil*

["Plants injured" means those that showed phloem injury or heart rot. In many cases most of the plants died and only the indicated numbers showed the types of injury under consideration]

Period of exposure (hours)	Temperature of freezing chamber (°C.)		Period between exposure and observation	Plants frozen	Plants injured	Type of injury
	Range	Average				
			Months	*Number*	*Number*	
5	−5 to −11	−6.6	6	10	1	Slight phloem injury and heart rot.
7	−5 to −18	−8.2	6	5	2	One plant, both heart rot and phloem injury. One plant, phloem injury only.
4	−8 to −17	−11.6	4	10	1	Phloem injury and heart rot.
3	−8 to −14	−11.6	4	5	1	Phloem injury.
6	−8 to −17	−13.8	4	5	5	Do.
7	−8 to −20	−14.5	5	5	1	Do.
4	−5 to −21.4	−17	6	10	3	Do.
2	−18 to −24	−21	4	5	3	Do.
3	−18 to −24	−21	4	10	2	Do.
Total				65	19	

The data presented in Table 1 show that in these experiments phloem injury was more often produced than heart rot. They also show that both types of injury were produced when the plants were exposed to average temperatures of −6.6°, −8.2°, and −11.6° C. for 5, 7, and 4 hours, respectively. On the other hand, phloem injury alone was evident in plants frozen at average temperatures of −11.6°, −13.8°, −14.5°, −17°, −21°, and −21° for 3, 6, 7, 4, 2,

and 3 hours, respectively. It is apparent that the types of injury under consideration can be produced over a considerable range of temperature and time of exposure. Characteristic phloem injury is shown in Figure 1, A–C. In some cases 100 per cent of the plants developed phloem injury, but more frequently the percentage of injured plants was much smaller. A total of 29.2 per cent of the plants used in these tests eventually showed either one or both types of injury. Why one type of injury was produced in some cases and both types in others is not clear from the tests made. All the plants frozen showed more or less injury to the stems and buds.

In Table 2 are shown the results of freezing plants taken from the same lot as those used in the experiments listed in Table 1, but in

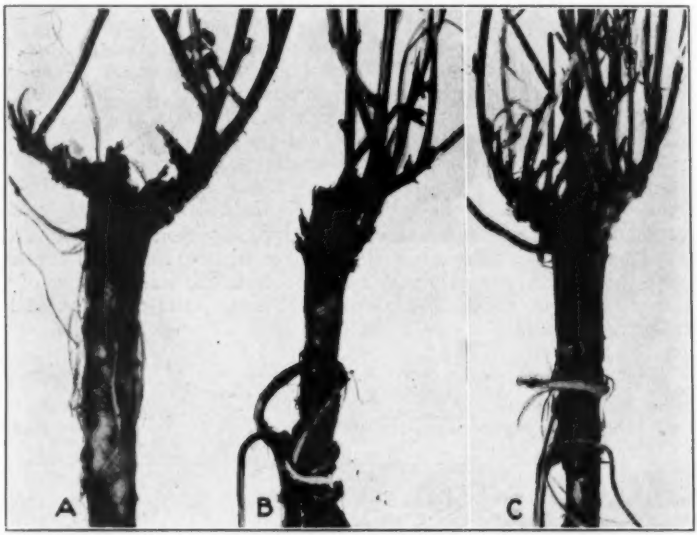

FIGURE 1.—A–C, Phloem injury of Kansas common alfalfa plants produced by artificial freezing. The plants were grown from seed sown in the field in the spring. They were transplanted to 8-inch pots December 1, 1926, and kept in the greenhouse until January 25, 1927, when they were exposed for seven hours to an average temperature of −14.5° C. After that they were kept in the greenhouse until July 1, when the photograph was taken. The blackening of the crown and upper part of the taproot illustrates what is designated in this paper as phloem injury. In some seasons lesions of this type are very common on alfalfa plants following severe winter injury. About one-half natural size

this case the plants were not brought into the greenhouse until they were needed for the freezing tests, which were conducted in February, 1927. These plants were subjected to freezing temperatures while in the field, but they showed no evidence of injury when removed from the soil, nor did the controls after growing in the greenhouse for several months. Two lots of 10 plants each were frozen for 1½ and 3 hours at average temperatures of −14.6° and −14.9° C., respectively. In these instances the roots were not planted in soil but were exposed to the temperature of the freezing chamber without any protection. The plants exposed for 3 hours were very seriously injured and most of them died. One of the plants which survived showed phloem injury after growing for four months in the greenhouse. On the other hand, although the plants frozen for 1½ hours

were not very seriously damaged, one of them showed some phloem injury. (Fig. 2.)

The last experiment listed in Table 2 illustrates an instance in which phloem injury developed in two out of five plants frozen in soil in 8-inch pots for seven hours at an average air temperature of −14° C.

TABLE 2.—*Phloem injury produced as a result of freezing 9-months-old Kansas common alfalfa plants on February 25, 1927, immediately after bringing them from the field*

"Plants injured" means those that showed phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Period of exposure (hours)	Temperature of freezing chamber (°C.)		Frozen in or out of soil	Period between exposure and observation	Plants frozen	Plants injured
	Range	Average				
				Months	*Number*	*Number*
1½	−13 to −16	−14.6	Out	4	10	1
3	−13 to −17	−14.9	do	4	10	1
7	−8 to −17	−14	In	4	5	2
Total					25	4

In Table 3 are summarized the results obtained by freezing Kansas common and Grimm alfalfa plants under various conditions at Madison, Wis. The plants, which were from spring-sown plots, were brought into the greenhouse at the time the experiments were conducted. Twenty-five plants of each variety were placed in soil in specially constructed cans and lowered into the freezing chambers, in which they were exposed to different temperatures for various periods of time. The plants were then replanted in soil, each variety being placed in a different pot. In some cases the pots were held outside of the greenhouse until danger of freezing became imminent, at which time they were placed in a cool greenhouse. Other pots were held in the greenhouse throughout the growing period. In the last two tests listed the plants were frozen in ice by placing the tops, including the crowns and 1 or 2 inches of the upper part of the taproots, in water, which was then frozen. The temperature of the water or ice was obtained at intervals by means of a thermocouple placed in the water along with the plants. The period of time listed in Table 3 includes that necessary to freeze the water, which

FIGURE 2.—Phloem injury on a taproot from the same lot of alfalfa as that illustrated in Figure 1. However, the plant was removed from the soil and brought into the greenhouse February 25, 1927, just before it was exposed for 1½ hours to a temperature of −14.6° C. After the exposure it was planted in soil in the greenhouse and kept there until June 23, when it was photographed. One-half natural size

was usually 8 to 10 hours. After the plants had been frozen for the desired length of time the ice was allowed to thaw at room temperature, and the plants were then set in soil in pots. Observations taken after 40 days, and 17 and 32 days in the last two instances, showed that a small percentage of the plants had phloem injury, heart rot, or both. A total of 6.9 per cent of the plants showed these types of injury. Many of the plants in some tests were killed outright, others were uninjured, and still others suffered various degrees of injury, as shown by the condition of their tops. The typical lesions under consideration were usually found in the few surviving plants in a pot where most of the plants had died. These lesions were found on both the Kansas common and Grimm plants and were produced when the plants were frozen in the soil or in ice for 24 to 48 hours. (Fig. 3.)

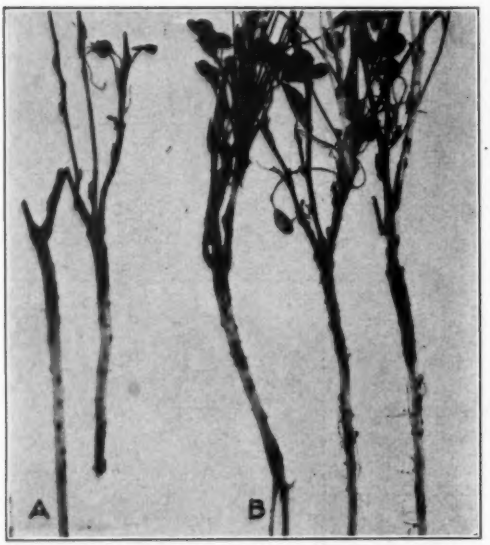

FIGURE 3.—Phloem injury produced by freezing 5-months-old alfalfa plants in soil for 24 hours at an average temperature of −11° C., in a refrigerating machine at Madison, Wis. The two plants (A) and the three (B) are of the Kansas common and Grimm varieties, respectively. These plants were kept in the greenhouse after freezing for 35 days before being photographed. In most cases the entire crowns and upper parts of the taproots are involved. The plant at the left in B shows a distinct band of dead tissue just below the crown. About two-fifths natural size

In Table 4 are shown the conditions under which typical phloem injury was produced in 12 out of 80 small alfalfa plants of the Kansas common variety, some of which were grown in the greenhouse and the remainder in the field. These plants were removed from the soil, washed, and placed tops down in a chamber cooled with ice and salt to temperatures ranging from −5° to −15° C. for different periods of time. After being frozen the plants were set in soil in a bed in the greenhouse. The observations made after a month, or in the last two instances after 21 and 7 days, respectively, showed typical phloem injury in 15 per cent of the plants. (Fig. 4.) No injury was evident in the unfrozen controls.

Although phloem injury and heart rot have developed occasionally in plants frozen in other experiments, the above-cited examples are sufficient to show something of the range of conditions under which these troubles may develop.

TABLE 3.—*Phloem injury and heart rot produced as a result of freezing 5-months-old alfalfa plants immediately after bringing them from the field at Madison, Wis.*

["Plants injured" means those that showed heart rot or phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Variety	Period of exposure	Temperature of freezing chamber (°C.)		Frozen in soil or in ice	Period between exposure and observation	Plants frozen	Plants injured	Type of injury
		Range	Average					
	Hours				*Days*	*Number*	*Number*	
Kansas common	48	−8 to −13	−11	Soil	40	25	1	Phloem injury.
Grimm	48	−8 to −13	−11	do	40	25	1	Do.
Kansas common	48	−8 to −13	−11	do	40	25	2	Do.
Do	24	−8 to −13	−11	do	40	25	1	Do.
Do	24	−8 to −13	−11	do	40	25	1	Do.
Grimm	24	−12 to −16	−14	do	40	25	1	Do.
Kansas common	48	−12 to −16	−14	do	40	25	1	Do.
Do	24	−12 to −16	−14	do	40	25	2	Heart rot and phloem injury.
Grimm	24	−12 to −16	−14	do	40	25	3	Phloem injury.
Do	39	[a] 0 to −10		Ice	17	25	4	Do.
Do	27	[a] 0 to −11		do	32	25	2	Do.
Total						275	19	

[a] Temperature in ice as determined by a thermocouple.

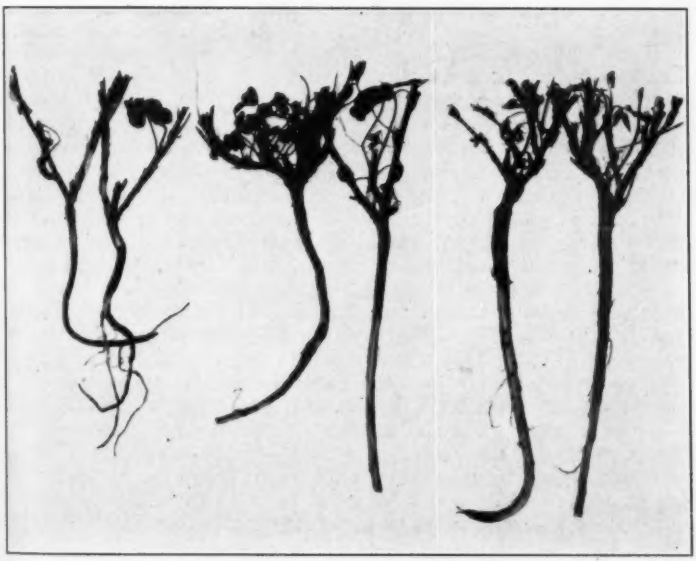

FIGURE 4.—Phloem injury developed by freezing in 3-months-old Kansas common alfalfa plants grown at Manhattan, Kans. On November 25, 1927, plants were brought from the field and frozen at temperatures varying from −11 to −15° C. for 15 to 25 minutes before being set in soil in the greenhouse. They were kept in the greenhouse until photographed on December 15, 1927. In most plants the injury is evident at varying distances below the crown. About two-fifths natural size

TABLE 4.—*Phloem injury produced as a result of freezing 3-months-old Kansas common alfalfa plants removed from soil and frozen during 1927–28*

["Plants injured" means those that showed phloem injury. In many cases most of the plants died, and only the indicated numbers showed the type of injury under consideration]

Source of plants	Period of exposure	Temperature (° C.)		Period between exposure and observation	Plants frozen	Plants injured
		Range	Average			
	Minutes				*Number*	*Number*
Greenhouse	15	−13.4 to −15	−14.2	1 month	10	2
Field	15	−11 to −13	−12	do	15	2
Do	20	−11 to −13	−12	do	15	2
Do	25	−11 to −13	−12	do	15	3
Greenhouse	40	−5 to −7	−6	21 days	15	2
Field	10		−11.5	7 days	10	1
Total					80	12

FREEZING PLANTS IN THE FIELD

It was thought that perhaps winter-injury lesions could be produced more readily if the plants were frozen as they stood in the soil by applying the cold from above only, thereby more nearly reproducing natural conditions. With this in mind, a number of experiments were conducted in which plants were frozen as they stood in the field, and in a few instances in the bed in the greenhouse, by placing over them a sheet-iron tank containing different proportions of ice and salt as described under the discussion of methods. The plants were growing in rows, and the chamber covered 10 to 15 plants. Temperatures of from −5° to −15° C. could be obtained in this way. The temperature records were taken by means to two maximum and minimum thermometers whose bulbs were placed among the crowns of the plants, one at the end and the other at the center of the group of plants covered by the chamber. In this manner the crowns and the upper part of the taproots of plants about 1½ years old were subjected to various degrees of cold for different periods of time. The tests were conducted during the summer, when the plants could be most readily frozen and when microorganisms would be most active. (The possible relation of microorganisms to the development of these lesions is discussed later.) Since nonhardened greenhouse plants developed the typical injuries, there seemed to be no reason why this work in the field could not be conducted as well in the summer as in the winter.

The crowns and the upper part of the taproots of many of the plants frozen in these experiments died outright. Usually the plants at each end of the row included beneath the chamber died or were severely injured, while those at the center were only slightly affected, and the plants between them showed various degrees of injury as indicated by the appearance of the tops. In no case, however, was typical phloem injury or heart rot produced. A photograph of a plant frozen in this manner is illustrated in Figure 5. However, in this case the freezing was so severe that the root was frozen entirely through. This was typical of what happened in many cases. As it seemed impracticable to produce by this method the lesions so commonly seen in plants frozen under natural conditions, this type of experiment was discontinued. Perhaps further trials at different seasons of the year might have yielded the desired results.

SOME HISTOLOGICAL CHANGES IN FROZEN ROOTS

Jones (*2*) recently published a paper giving a detailed discussion of the histological changes in alfalfa roots due to winter injury. His studies were all made on material collected from the field. While conducting the freezing experiments at Madison, Wis., details of which are recorded in Table 3, Jones and the writer studied some of the plants frozen under known conditions in order to compare them with the field material studied previously by Jones. Portions of the taproots just below the crown were fixed, embedded in paraffin, sectioned, and stained by the method described by Jones (*2*). Plants just taken from the freezing chambers as well as those which had been growing for a month in the greenhouse after being frozen and which

FIGURE 5.—Plant frozen by placing a can containing ice and salt over it as it stood in a bed in the greenhouse. The minimum temperature to which it was exposed was −13.5° C., and the time of exposure was 23 hours. The crown and the upper part of the taproot were frozen entirely through. The root was halved longitudinally, and the exterior (A) and interior (B) views are shown. About three-fourths natural size

showed typical phloem injury and heart rot were studied. Figures illustrating different types of injuries are given herein.

In Figure 6 is shown a cross section of the root near the crown of a 5-months-old Kansas common alfalfa plant frozen for 24 hours at a temperature varying from −12° to −16° C. (average about −14°) as it appeared when it came from the freezing chamber. The rays are badly split in both the xylem and the phloem, and the phloem parenchyma cells just beneath the exterior cork covering are more or less broken apart. Some roots of the same lot that had been subjected to the same conditions showed few or no rifts in the rays. In some cases the splitting was largely confined to wood rays, while in others only the phloem rays were affected. The amount of splitting also varied greatly. However, in extreme cases both the wood and phloem

rays were split. The cambium cells were frequently collapsed. There was no unusual staining reaction at this time, the sections taking the stain as did healthy tissue of unfrozen control plants, sections of which were prepared and studied at the same time. The splitting off and sloughing away of the outer layer of the upper part of the taproot and crown of young plants when frozen in the field are of very common occurrence. The manner in which such a condition is brought about is evident in the section shown in Figure 6. In some

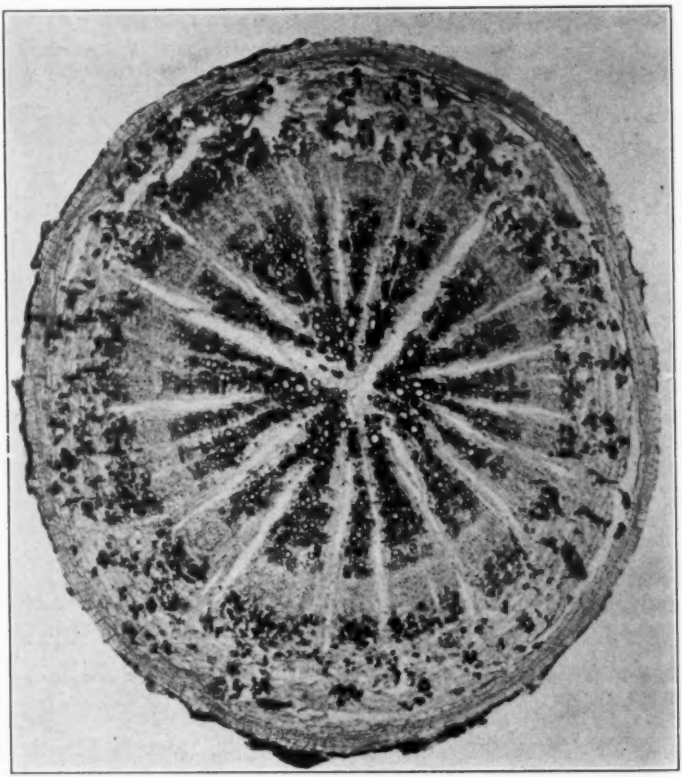

FIGURE 6.—Cross section of a root of a 5-months-old Kansas common alfalfa plant frozen in a pot of soil for 24 hours at an average temperature of −14° C. Note the rifts along the rays (light streaks) as they appeared when the root was taken from the freezing chamber. Compare with sections of roots from the same lot illustrated in Figures 7, A and B, and 8, A. × 4½

sections this layer is found to have been entirely loosened from the phloem parenchyma beneath.

In Figure 7, A, is shown a cross section of one of the roots of the same lot as that discussed above. This root had been planted in soil and grown for 40 days before it was photographed. It shows typical heart rot without any apparent phloem injury. A portion of this root was infiltrated with paraffin, sectioned, stained, and photomicrographed. It is reproduced as Figure 7, B, and shows that a

considerable amount of splitting of the wood rays was caused by the freezing. There appears to be some injury in the phloem parenchyma, especially near the side rootlet. Injury in the xylem as well as the phloem is denoted by the deeper stain which has photographed black. The cambium in this plant was to all appearance uninjured, and the cork layer remained intact throughout.

A photomicrograph of another root of the same lot is shown in Figure 8, A. This root also had heart rot as well as phloem injury. Much splitting in the wood rays is evident, and the injured cells

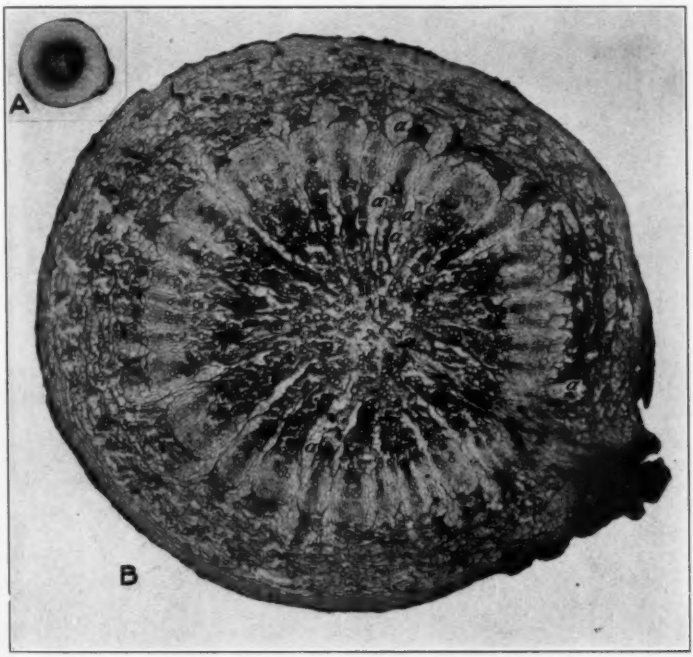

FIGURE 7.—A, Cross section of an alfalfa root from the same lot as that in Figure 6. The plant had grown in the greenhouse for 40 days before being sectioned. Typical heart rot had developed in this root as indicated by its dark center. × 5. B, Cross section of the same root as in A, showing in more detail the structure of the root. The rifts in the wood rays are still evident, and many of the cells have died from the effect of freezing and take the stain more deeply than the living cells. There was also a limited amount of phloem injury near the side rootlet, as indicated by the darker stain. Some of the openings resulting from the freezing, a few of which are indicated by the letter *a*, have been closed by the formation of new cells within them. The outline of the original opening is still evident. Compare with Figure 8, B, which shows similar rifts still remaining open due to the fact that the surrounding cells were killed by freezing. × 32

largely near the cracks took the stain more deeply. The greatest injury in this root is in the phloem, as indicated by the dark-staining area. Many of the phloem cells have collapsed and are badly disorganized. A meristematic layer has been laid down in the phloem which separates the dark-staining injured tissue from the uninjured portion of the phloem nearer the cambium. This layer is not equal in thickness and undoubtedly is not such an effective barrier as was furnished by the cork layer originally present on the outside of the root. The dead tissue outside of this wound periderm was decaying

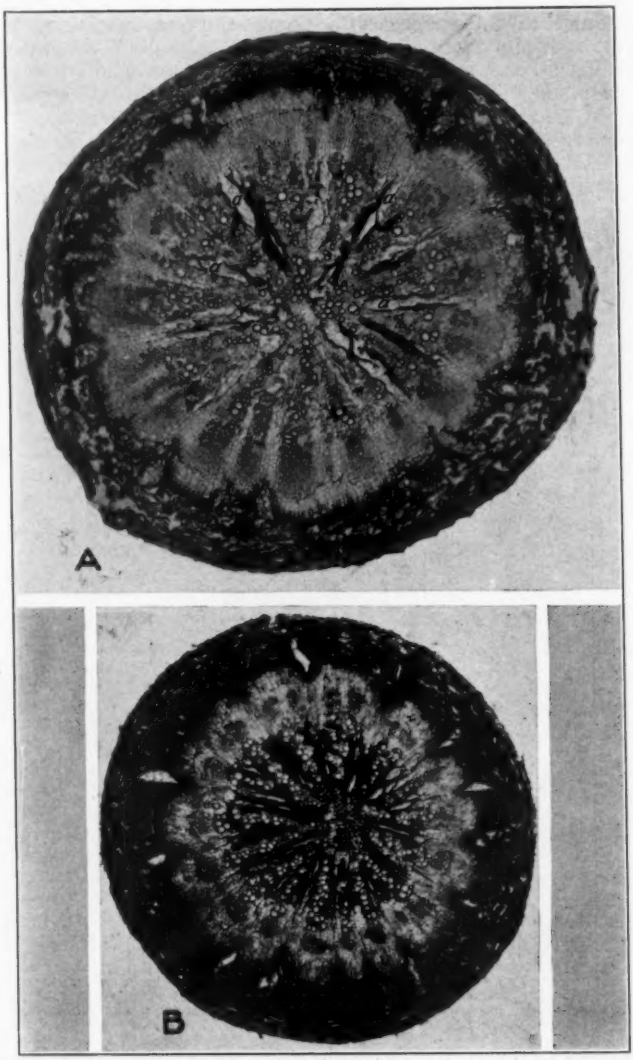

FIGURE 8.—A, Cross section of a root from the same lot of alfalfa as that shown in Figures 6 and 7, A and B, taken 40 days after freezing. Here the phloem seems to have suffered most, as a large part of the cells were killed outright. There is some splitting of the wood rays bordered with dead cells as well as some dead cells in the wood which have stained dark. This figure shows the appearance of the interior of roots affected with phloem injury as illustrated in Figures 3 and 4. Note also the healed cracks at the points indicated by *a*. × 32. B, Cross section of an alfalfa root of about the same age as that shown in A and frozen under natural conditions at Manhattan, Kans., during the winter of 1927–28. Both phloem injury and heart rot followed the splitting of the rays and death of the cells. Much of the phloem was killed by the low temperature, as indicated by the black-staining area, in the outer part of the section. Many of the largest rifts are surrounded with dead (black) cells, and there is little if any evidence of the closing of these openings by the formation of new cells. Considerable meristematic activity that had taken place resulted in the formation of a wound periderm just inside of the dead portions of the phloem. × 19

and would no doubt eventually have sloughed off. In the meantime this dead tissue is open to attack by various soil fungi and bacteria, some of which may be weak parasites, and once having established themselves in the dead tissue, can find their way through the weakened or even incomplete periderm beneath and hasten the death of the plant.

A section of a root showing more severe heart rot as well as phloem injury is shown in Figure 8, B. Here the frost cracks both in the phloem and xylem are surrounded by the deep-staining dead cells. As in Figure 8, A, the inner part of the phloem is separated from the dead outer portion by a heavy meristematic layer. This outer tissue eventually sloughs off, the dead central xylem decays, and the result is a root whose crown and upper part of the taproot form little more than a thin-walled hollow cylinder with a roughened exterior, resembling the root previously illustrated by Weimer (*6, fig. 2, A and B*).

It having been observed that the heart rot and phloem injury were usually found in plants that showed more or less ray splitting and tearing apart of parenchymatous cells, the question arose as to whether such splitting was necessarily associated with the production of such symptoms. To obtain information on this point, roots of plants that had been frozen by different methods were sectioned. It was soon learned that plants removed from the soil and frozen quickly did not always show splitting, at least not to the same extent as did similar plants frozen slowly in soil. Young plants in soil in pots withstood an air temperature of −10° C. or thereabouts (soil temperature not recorded) for 48 hours, but they were killed in 15 to 20 minutes at that temperature when removed from the soil and then frozen. It seems probable that, when the plants are cooled slowly as they are when frozen in soil, large ice crystals are formed between the cells in certain local areas—especially where the cell walls are thin and the water content high, as in the rays and phloem parenchyma—and the cells are broken apart. On the other hand, when freezing is rapid, ice crystals are formed comparatively uniformly throughout the tissue and the cells are killed without the localized splitting and tearing. At least this explanation seems plausible in the light of Wiegand's studies on the formation of ice crystals in plant tissue (*7*).

ASSOCIATION OF MICROORGANISMS WITH WINTER-INJURY LESIONS

Isolations were made from a large number of plants affected with phloem injury and heart rot from the field and the greenhouse, and usually a species of Fusarium, bacteria, or both were obtained. More rarely *Rhizoctonia solani* was isolated. However, the same species of Fusarium was seldom obtained from different lesions. No one organism could be isolated very consistently from such decaying tissues. It seemed probable, therefore, that these microorganisms associated with the lesions occurring in roots subjected to freezing were simply saprophytic organisms living in the frozen tissue. This supposition was substantiated by the fact that no such lesions were ever found on unfrozen control plants or plants only slightly frozen.

In order to determine the pathogenicity of some of the organisms isolated, the following experiment was conducted: Young alfalfa plants of the Kansas common variety growing in the greenhouse were

removed from the pots and frozen for 10 and 15 minutes at a temperature of −12° C., and then they were planted in soil in 8-inch pots and held in the greenhouse. The following treatments were used:

(1) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in soil steamed for four hours without pressure and not infested with the organisms being tested. For each lot of plants 4 pots were used, making a total of 12 pots containing 60 plants.

(2) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in steamed soil infested with Fusaria or bacteria isolated from typical phloem-injury lesions. For each organism 1 pot was used with each lot of plants, or a total of 24 pots containing 120 plants.

(3) Plants frozen for 15 minutes and unfrozen controls were planted in steamed soils infested with Fusaria or bacteria as above, but in addition some of the inoculum was inserted in a wound in the taproot just below the crown of each plant. For each organism 1 pot was used with each lot of plants, or a total of 16 pots containing 80 plants.

(4) Plants frozen for 10 and 15 minutes and unfrozen controls were planted in soil not steamed and not infested. For each lot of plants 4 pots were used, or a total of 12 pots containing 60 plants.

In the experiment there were used 320 plants, of which 80 were frozen for 10 minutes, 120 were frozen for 15 minutes, and 120 were unfrozen. Likewise 120 plants were left uninoculated, and 200 were inoculated in wounds or planted in infested soil.

The organisms used consisted of five species of Fusarium and three species of bacteria. These were chosen as being representative of the organisms most commonly isolated. The Fusaria were grown on sterilized oats in flasks and the bacteria on nutrient agar. As some of the oats as well as the fungus mycelium was added to the soil infested, a large amount of inoculum was present in all cases.

The experiment was initiated on April 6, 1928, and notes were taken on the frozen plants on April 27 and on the unfrozen controls on May 16. Although many of the frozen plants were so severely injured that they died, some grew fairly well. In no case did any typical phloem injury or heart rot such as has been described and illustrated develop. Neither was there any evidence that the organisms used in the inoculations were capable of attacking unfrozen plants. Some of the frozen roots had decayed somewhat, especially at the lower ends, and in a few cases the Fusarium placed in the soil was reisolated from the decaying plant. However, here again no one organism could be consistently isolated from these roots. The plants in the steamed uninfested soil as well as in the unsteamed soil contained Fusaria and bacteria.

This experiment was duplicated, using twice as many smaller plants from the field. However, the frozen plants were so severely injured that many of them did not recover. In this experiment, as in the previous one, unfrozen control plants failed to show any evidence of infection.

The results of these experiments indicate that the microorganisms associated with phloem injury and heart rot are not parasitic on the unfrozen plants. Apparently these organisms are capable of living in badly frozen roots and perhaps can aid in their ultimate disintegration. Just how much progress such fungi and bacteria can make in moderately injured tissue has not been determined.

Typical phloem injury and heart rot have been found in plants in the field early in the spring when the temperature was still so low that it seems probable that the microorganisms in the soil had not yet

become very active. On the other hand, these troubles are more commonly found in the field later in the season after ample opportunity has been afforded for the action of microorganisms. This does not mean, however, that microorganisms are a factor in the development of these typical lesions. The collapse of the dead and injured cells is probably sufficient to produce the type of lesions so commonly found in frozen alfalfa plants.

HEALING OF OPENINGS PRODUCED IN RAYS BY FREEZING

It has been pointed out that splitting of the rays of alfalfa roots is a very common result of freezing and that in some instances most if not all of a given lot of plants may show such splitting. The amount of ray splitting in plants receiving the same treatment varies considerably. More or less splitting may occur unaccompanied by any evident injury. Frequently, however, many of the cells adjacent to the cracks are injured or even killed outright. Splitting alone probably would not usually be fatal to the plant, but splitting is so frequently accompanied by the killing of cells that it is hard to consider one without the other. In the present discussion, however, splitting is emphasized, since the presence of cracks and their healing were the chief points in mind when these studies were made.

It was observed by F. R. Jones that the roots of alfalfa plants collected during the winter and early summer frequently showed evidence of ray splitting, while many of those obtained from the same field later in the season did not. This same thing was observed subsequently by the writer while studying sections of varieties and strains of alfalfa to determine the comparative amount of ray splitting. It was apparent that many of these openings in the rays must be closed either by mechanical pressure, by the formation of new cells, by the enlargement of neighboring cells, or perhaps by a combination of all of these methods.

Portions of the taproots about 0.5 cm. in length taken about 2 cm. below the crown of 2 to 5 plants of each of 16 varieties of alfalfa were collected on January 31, April 16, June 1, June 27, and August 1, 1928. These specimens were placed at once in a fixer, infiltrated with paraffin, sectioned, and stained. The method of staining was similar to that described by Jones (*2*). The varieties or strains of alfalfa used were as follows: Grimm, Cossack, Hardigan, Kansas common, Turkestan, Lebeau, Argentine, Dakota, Utah, South African, Spanish, Italian, Smooth Peruvian, Hairy Peruvian, Arizona, and India. The plants were growing in rows in a small level plot on a fairly uniform well-drained soil at Manhattan, Kans. All the plants were of the same age, the seed having been sown in April, 1927.

The data accumulated in these studies are given in Table 5. It would have been desirable to use more plants of each group, but they were not available. All the plants of the India variety which survived the winter were used in the first three collections. Although the number of plants of each variety used each time was rather small, a total of nearly 300 plants was studied.

TABLE 5.—*Amount of splitting in rays of roots of different varieties and strains of alfalfa collected on different dates in 1928 and the progress of healing of the openings*

[In some collections healing is indicated where no splitting was present, this is due to the fact that the cracks were closed during the healing process]

Variety	Jan. 31						Apr. 16						June 1						June 27						Aug. 1					
	Number of plants	Plants with the indicated amount of ray splitting				Plants showing healing	Number of plants	Plants with the indicated amount of ray splitting				Plants showing healing	Number of plants	Plants with the indicated amount of ray splitting				Plants showing healing	Number of plants	Plants with the indicated amount of ray splitting				Plants showing healing	Number of plants	Plants with the indicated amount of ray splitting				Plants showing healing
		None	Slight	Moderate	Severe			None	Slight	Moderate	Severe			None	Slight	Moderate	Severe			None	Slight	Moderate	Severe			None	Slight	Moderate	Severe	
Grimm	4	0	4	0	0	0	5	0	5	0	0	0	4	0	4	0	0	0	4	4	0	0	0	2	4	4	0	0	0	0
Cossack	3	0	2	1	0	0	4	0	2	2	0	0	4	2	1	1	0	0	2	2	0	0	0	2	3	3	0	0	0	1
Hardigan	4	0	2	2	0	0	5	0	3	2	0	0	3	2	1	0	0	0	2	1	1	0	0	0	3	3	0	0	0	1
Kansas common	4	1	1	2	0	0	3	0	1	2	0	0	4	1	2	1	0	3	4	3	1	0	0	3	4	3	1	0	0	2
Turkestan	4	1	3	0	0	0	2	1	1	0	0	0	4	2	2	0	0	0	4	4	0	0	0	0	2	2	0	0	0	2
Lebeau	5	2	3	0	0	0	4	3	1	0	0	0	4	2	2	0	0	1	4	4	0	0	0	3	3	3	0	0	0	1
Argentine	4	0	3	0	1	0	5	0	3	2	0	0	3	1	2	0	0	2	3	1	2	0	0	2	4	4	0	0	0	3
Dakota	4	1	3	0	0	0	5	0	5	0	0	0	4	1	3	0	0	3	4	3	1	0	0	2	4	4	0	0	0	1
Utah	5	0	1	4	0	0	5	0	2	3	0	0	4	0	3	1	0	2	4	4	0	0	0	4	4	4	0	0	0	3
South African	4	0	1	0	3	0	4	0	0	0	4	0	4	2	2	0	0	4	3	2	1	0	0	3	4	3	1	0	0	4
Spanish	5	0	0	0	5	0	5	0	2	0	3	1	2	1	1	0	0	1	2	2	0	0	0	1	4	4	0	0	0	4
Italian	4	0	3	1	0	0	4	0	4	0	0	0	4	1	3	0	0	4	4	1	3	0	0	4	3	1	2	0	0	2
Smooth Peruvian	2	0	0	2	0	0	4	0	2	1	1	0	2	1	0	1	0	2	3	1	1	1	0	3	4	2	2	0	0	4
Hairy Peruvian	4	0	1	0	3	0	5	0	3	0	2	0	2	0	1	1	0	0	4	3	1	0	0	4	4	3	1	0	0	4
Arizona	4	0	0	0	4	0	5	3	0	0	2	2	2	1	1	0	0	2	4	4	0	0	0	3	4	4	0	0	0	3
India	4	0	0	0	4	0	4	0	1	2	1	1	4	0	3	1	0	3												
Total	64	5	27	12	20	0	69	7	35	14	13	4	54	17	31	6	0	27	51	39	11	1	0	36	54	47	7	0	0	35

The data presented in Table 5 show some interesting comparisons. Expressed in percentages, 92.2 per cent of the plants showed ray splitting in January. The amount of splitting gradually decreased as the season advanced, being 89.8, 68.5, 23.5, and 13, respectively, for April 16, June 1, June 27, and August 1. The percentage of plants showing no evidence of splitting naturally increased as the rifts healed, starting at 7.8 per cent in January and increasing to 10.1, 31.5, 76.5, and 87 per cent on April 16, June 1, June 27, and August 1, respectively. Likewise the percentage of plants showing healing was greater at each collection with the exception of the last, starting at 0 in January and increasing to 5.8, 50, 70.6, and 64.8 per cent, respectively, in the different collections.

Not only did the total number of plants showing rifts in the roots decrease as the season advanced and healing progressed, but the amount of splitting also varied. For example, in the slightly split groups the percentages were 42.2, 50.7, 57.4, 21.6, and 13; in the moderately split ones they were 18.7, 20.3, 11.1, 2, and 0; and in the severely split ones they were 31.2, 18.8, 0, 0, and 0, on January 31, April 16, June 1, June 27, and August 1, respectively.

In Table 6 is given a set of figures derived from those in Table 5. These illustrate clearly the decline from January to August in the percentage of plants showing splitting as well as the increase in the amount of healing. This table also brings out more sharply the contrast between the different varieties in regard to the amount of splitting and the rate of healing. For example, all but four varieties—

Kansas common, Turkestan, Lebeau, and Dakota—showed splitting in 100 per cent of the plants examined in January. In April all but Turkestan, Lebeau, and Arizona showed splitting in 100 per cent of the roots studied. A rather decided change was noticeable, however, on June 1, at which time only four varieties—Grimm, Utah, Hairy Peruvian, and India—showed openings in all of the roots examined, and also on August 1, when only Kansas common, South African, Italian, Smooth Peruvian, and Hairy Peruvian showed any splitting at all.

TABLE 6.—*Percentage of roots of different varieties of alfalfa showing ray splitting and healing on different dates in 1928*

[Partial summary of figures presented in Table 5]

Variety	Percentage of plants showing ray splitting on—					Percentage of plants showing healing on—				
	Jan. 31	Apr. 16	June 1	June 27	Aug. 1	Jan. 31	Apr. 16	June 1	June 27	Aug. 1
Grimm	100	100	100	0	0	0	0	0	50	0
Cossack	100	100	50	0	0	0	0	0	100	33.3
Hardigan	100	100	33.3	50	0	0	0	0	0	33.3
Kansas common	75	100	75	25	25	0	0	75	75	50
Turkestan	75	50	50	0	0	0	0	0	0	100
Lebeau	60	25	50	0	0	0	0	25	75	33.3
Argentine	100	100	66.7	66.7	0	0	0	66.7	66.7	75
Dakota	75	100	75	25	0	0	0	75	50	25
Utah	100	100	100	0	0	0	0	50	100	75
South African	100	100	50	33.3	25	0	0	100	100	100
Spanish	100	100	50	0	0	0	20	50	50	100
Italian	100	100	75	75	66.7	0	0	100	100	66.7
Smooth Peruvian	100	100	50	66.7	50	0	0	100	100	100
Hairy Peruvian	100	100	100	25	25	0	0	0	100	100
Arizona	100	40	50	0	0	0	40	100	75	75
India	100	100	100			0	25	75		

The cracks had begun to close in three varieties—Spanish, Arizona, and India—by April 16, and in many others by June 1. In general the healing started first and progressed most rapidly in the least hardy varieties, such as the South African, Spanish, Italian, Smooth Peruvian, Hairy Peruvian, Arizona, and India. On the other hand, Grimm and Hardigan, representatives of the most hardy varieties, showed less evidence of healing than any of the others, although most of the roots of these varieties examined earlier had shown some splitting. The rifts in the more hardy varieties, however, were usually not so large, and there probably was less killing of adjacent cells; consequently many of the openings were closed without leaving any evidence of their having been present except possibly some enlarged ray cells resulting in some cases in a slight widening of the rays themselves. No healing was seen in the Turkestan roots until August 1. Lebeau, followed by Turkestan, appeared to have fewer rifts in the rays than any other variety studies.

Considerable tearing apart of cells of the phloem parenchyma was observed in many of the most severely split roots. This disruption of the cellular structure was often accompanied by the killing of the cells themselves. Rifts in this part of the root usually are comparatively small and are also frequently closed by the formation of new cells.

These histological studies failed to disclose any criterion that might serve to distinguish definitely between hardy and nonhardy varieties

of alfalfa. The investigations, however, were not exhaustive, as they dealt largely with the relative amount of splitting and did not include a detailed study of the proportion of the various kinds of tissues or other histological differences in the roots.

It having been shown that alfalfa roots frozen artificially as well as in the field may be more or less severely split, and that these openings may close up after a period of growth, it is of interest to look more closely at the nature of the healing process. A clear picture of severe splitting produced by artificial freezing as described elsewhere is presented in Figure 6. Microscopic study showed not only that the rays were split badly but also that many of the cells were killed, and it seems probable that the plant would have died or that after a period of growth it would have looked somewhat like that illustrated in Figure 8, B. There is, of course, no evidence of healing in this root, as it was sectioned at once after freezing.

One type of healing is evident in Figure 8, A and B, where a wound periderm has been laid down just under the dead portion of the phloem. Another type of healing is illustrated in Figures 7, B, and 8, A, where there are groups of cells in or adjacent to certain of the cracks (some of which are indicated by the letter *a*) that are larger than any of their neighbors. These, the writer believes, have been formed since the roots were frozen, in response to a wound stimulus. If this interpretation is correct, a considerable amount of healing can take place in a period of 40 days under good growing conditions. Other examples of rifts closed in a similar manner are illustrated in Figures 9 and 10.

A photomicrograph of the center of a cross section of the taproot of an alfalfa plant of the Arizona variety is shown in Figure 9, A. This is from a root included in the June 27 collection listed in Table 5. The black lines consisting of the dead cells adjacent to the rifts indicate where the frost cracks had been. The large parenchymatous cells have filled these openings, and the walls of the injured cells have been crushed together in such a manner that only the black lines are now visible. In some instances, as in Figure 9, A, *a*, and in other roots studied, the openings are not yet closed completely. On the other hand, in roots where healing is well advanced the cracks usually are no longer visible.

A section of another root of the Arizona variety collected on June 1, illustrated in Figure 9, B, has the rifts filled up entirely. This illustration is an enlargement of a portion of the center of the root shown in Figure 10, A and B, presented here to give a clearer picture of the character of the cells which fill in these cracks. These cells appear to be parenchymatous in character and to have originated either from ray cells contiguous to the openings or from parenchyma cells, which are abundant in the center of the root as well as in the wood itself. That there are cells all through these roots which are potentially meristematic in nature is evident by the fact that complete layers of wound periderm are laid down about many types of wounds. For example, a band of wound periderm several cells in thickness surrounds the dead central part of the root illustrated in Figure 10, A, and similar layers of cells are seen just beneath the injured phloem in Figure 8, A and B. Such cells, and especially those nearest the injured tissue, sometimes give a positive reaction for lignin when treated with phloroglucin and hydrochloric acid, and in other cases they give a positive

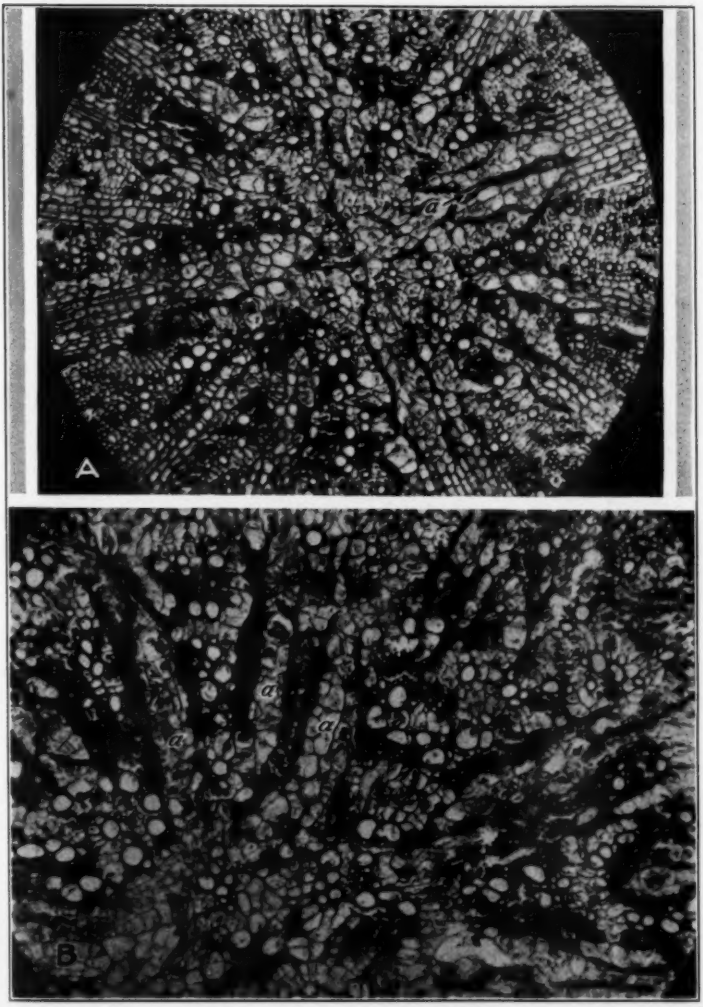

FIGURE 9.—A, Center of a cross section of a taproot of an alfalfa plant of the Arizona variety brought from the field on June 27, 1928. The black lines indicate the position of the frost cracks. The cells killed by the freezing were pressed aside as the open spaces were filled with new cells. One crack not entirely closed is evident in the upper right side of the picture as indicated by *a*. × 42. B, Portions of a section of another root of the same variety collected June 1, 1928. The rifts are completely healed in this root and many new cells have been formed. This is an enlargement of a portion of the section shown in Figure 10, B, presented here to show more clearly the character of the cells that fill in these cracks, *a*. × 81

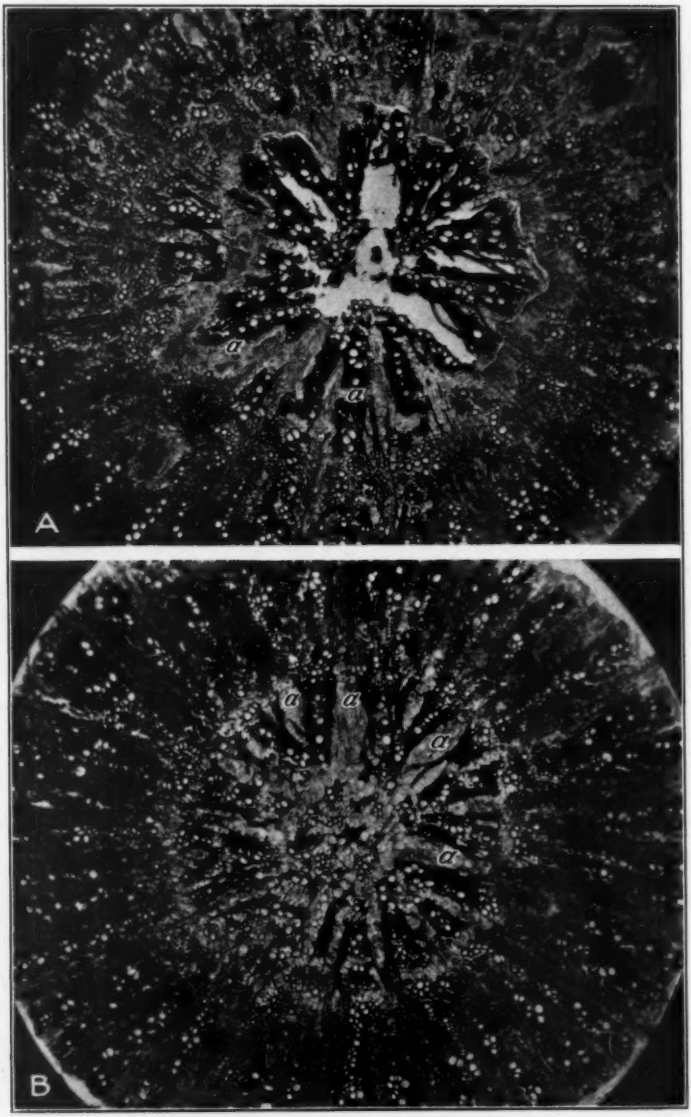

FIGURE 10.—A, Cross section of the center of an alfalfa root of the Arizona variety brought from the field on June 1, 1928. This figure shows a case of typical heart rot. The dead center of the root has been cut off from the live tissue outside by a band of wound periderm *a*. There was killing of cells as well as some splitting of rays outside of the dead central area. × 35. B, Cross section of the same root taken 2 inches below A. There is evidence of much splitting and killing of cells as in A, but the injury was not so severe and the cracks have been filled up completely by the formation of new parenchymatous cells *a*. This section was taken just below the portion of the root showing distinct heart rot. × 30

suberin reaction with Sudan III and are insoluble in 50 per cent chromic acid. They stain green with Pianeze III b, indicating the presence of lignin, suberin, or cutin in the walls. The dark lines bordering the healed rifts which have been described as crushed cell walls do not seem to have undergone much, if any, modification, as they stain like the surrounding parenchyma cell walls with all stains used. The large parenchymalike groups of cells which have closed up certain of the rifts have cellulose walls, as indicated by their staining reaction.

Figure 10, A and B, shows sections of the same taproot, but B was taken 2 inches lower than A, which came from near the crown. These sections show that the plant had a typical case of heart rot in the region of the crown, the tissues at the center having been so badly frozen that they could make no recovery. Two inches below, however, although the injury appears to have been quite severe, enough cells survived to initiate new growth, and the cracks were filled completely.

The data presented here seem to justify the following general conclusions: (1) A large percentage of the roots of alfalfa plants growing in climates where the temperature falls below −3° C. or thereabouts for extended periods may show slight to severe splitting in the rays each winter and spring due to freezing; (2) as the season advances, fewer roots show moderate or severe splitting, and even those exhibiting slight rifts decrease as the cracks almost, if not entirely, disappear by the end of the growing season; (3) some slight ray rifts appear to close without leaving any trace of their presence, and others leave distinct and clear-cut evidence that large openings have been entirely closed by the formation of new cells; and (4) large dead areas in either the phloem or the center of the root are cut off from the living tissue beneath by meristematic activity. Much variation in the amount of splitting and the rate of healing even under the same climatic conditions must be expected. These will vary with the age of the roots, the degree of hardening, the hardiness of the variety, and other factors. Jones (*2*) pointed out that the ability of alfalfa roots to heal their injuries is greater the first year than in subsequent years.

SUMMARY

Many alfalfa plants of the Kansas common and Grimm varieties were frozen by different methods and for different lengths of time in order to learn something of the manner in which certain types of root injuries that are so common in the field are produced.

The results of these investigations have confirmed the belief of others as well as of the writer that the cracked and decaying conditions of the crown and of the upper part of the taproot of so many alfalfa plants is due to freezing.

The terms "phloem injury" and "heart rot" are used to designate the injury to those parts of the root lying outside of and inclosed by the cambium, respectively. Most of the plants frozen either died outright or recovered without showing any very striking external evidence of injury to the roots. A certain percentage of the plants, however, did develop the lesions described in this paper as phloem injury and heart rot in one to several weeks after they were frozen.

Both phloem injury and heart rot developed in potted alfalfa plants frozen in soil at average temperatures of −6.6°, −8.2°, and

−11.6° C. for 5, 7, and 4 hours, respectively. Phloem injury alone was apparent macroscopically in plants frozen at −11.6°, −13.8°, −14.5°, −17°, −21°, and −21° C. for 3, 6, 7, 4, 2, and 3 hours, respectively. The plants frozen were grown in the field for about eight months and then transferred to the greenhouse. As they were not frozen at once, they were not in a hardened condition.

Phloem injury developed in plants brought from the field and frozen at −14.6° and −14.9° C. for 1½ and 3 hours, respectively, before being set in soil in the greenhouse. The same type of injury developed in 2 out of 5 plants of the same lot subjected to an average temperature of −14° C. for 7 hours. In this case the plants were set in soil in an 8-inch pot before they were exposed to the low temperature.

Phloem injury, heart rot, or both became evident in 19 out of 275, or 6.9 per cent, of the young alfalfa plants frozen in soil at Madison, Wis., in 40 days or less.

Phloem injury was produced in 3-months-old alfalfa plants by removing them from the soil and subjecting them to average temperatures of −14.2°, −12°, −12°, −12°, −6°, and −11.5° C. for 15, 15, 20, 25, 40, and 10 minutes, respectively.

Attempts to produce the typical phloem injury or heart rot in plants in the field in the summer by covering them with a can containing ice and salt mixture were unsuccessful. The plants could be killed readily by this means, but they either died or recovered without developing the typical winter-injury lesions on the roots.

Histological studies of some of the frozen roots showed that a typical splitting apart of the cells, especially along the rays of the root, usually was present. This tearing apart and the death of the cells in localized areas due to freezing, followed by their complete collapse and decay, result in the formation of the lesions described as phloem injury and heart rot. The typical splitting in artificially as well as in naturally frozen plants is illustrated.

Microorganisms are commonly associated with the typical winter-injury lesions under discussion. The parasitism of five species of Fusarium and three of bacteria isolated from typical phloem injury and heart rot was tested. In no case did they infect unfrozen plants. It seems clear that these associated microorganisms are for the most part at least saprophytes or at best very weak parasites.

Histological studies of the roots of nearly 300 plants of 16 different varieties or regional strains of alfalfa have shown that the rays are more or less severely split during the winter. If the injury is not too extreme the cracks formed are practically all closed during the following growing season.

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MAGNESIUM AND CALCIUM REQUIREMENTS OF THE TOBACCO CROP[1]

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INTRODUCTION

Through the use of improved methods, plant physiologists are now finding that the mineral nutrition of the plant is more complex than has been supposed and that apparently the list of indispensable elements must be considerably enlarged. Moreover, it is becoming apparent that under some conditions certain elements that have long been recognized as essential to normal growth but regarded as of no special significance in practical fertilizer usage must in fact be added to the soil if the best results are to be obtained. On the other hand, there is a definite trend in the fertilizer industry, largely for economic reasons, toward the use of relatively pure chemicals intended to furnish in highly concentrated form and comparatively free from "filler" or supposedly inert material the three elements, nitrogen, phosphorus, and potassium, which have been generally considered as furnishing a complete fertilizer. Mixtures of potassium nitrate and an ammonium phosphate or of ammonium nitrate and a phosphate of potassium are examples of a fertilizer of this sort. Experiments and observations discussed herein seem to indicate, however, that on some of the important tobacco soils the use of such materials alone can not be relied upon to give satisfactory results. The chief difficulty seems to be that the three elements mentioned do not under the circumstances constitute a complete fertilizer rather than that there is any failure of these elements in the forms used to function normally. Apparently lack of magnesium and calcium in the mixture is involved.

In a preliminary paper published in 1923 (*1*)[2] it was shown that on certain soils, when no magnesium is supplied in the fertilizer or from other sources, a distinctive chlorosis is likely to develop in the leaves of the tobacco plant, due to a breaking down of the chlorophyll. It is the purpose of the present paper to give somewhat more detailed information concerning the magnesium requirements of the tobacco crop and methods for supplying them and to make available the results of preliminary observations on the need of also supplying calcium in the tobacco fertilizer on certain soils. Little in the way of a background for the problems involved can be derived from accumulated experience in fertilizer usage or from the immense amount of ferti-

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[2] Reference is made by number (italic) to "Literature Cited," p. 168.

lizer experimentation which has been carried out with tobacco and other crops, because the fertilizers and fertilizer materials used almost invariably have been of a more or less complex chemical composition. For example, it is obvious that as long as superphosphate is an important constituent of the fertilizer the plant is not likely to show evidence of an insufficient supply of calcium. Similarly, many organic materials of plant or animal origin which have been widely used as components of the complete fertilizer contain notable quantities of both calcium and magnesium It should be kept in mind also that continued use of such materials over a period of years tends to set up in some soils a limited and essentially temporary reserve of calcium and magnesium which otherwise would not be present.

MINIMUM MAGNESIUM REQUIREMENTS OF TOBACCO

In the present connection the expression "minimum magnesium requirements" has reference to the minimum content of magnesium in the plant which will serve to prevent the appearance of visible symptoms of magnesium hunger, commonly spoken of as sand drown. The minimum quantities of magnesium required to prevent these symptoms, of course, would not necessarily be sufficient for the maximum growth of the plant.

The symptoms of magnesium hunger in tobacco have been described in the paper mentioned above (*1*). In other plants the symptoms are more or less similar to those observed in tobacco, but in general the trouble is not so readily diagnosed. In corn the lower blades of the plant show a striped effect, due to the breaking down of the chlorophyll in the areas between the veins. There is a general similarity in the distinctions between magnesium hunger and potassium hunger to those observed in tobacco. In soybeans the leaf presents an appearance clearly suggestive of that seen in tobacco. The tendency of the lower leaves to shed, however, interferes with the full development of deficiency symptoms. Cotton presents very striking symptoms of magnesium deficiency. In the initial stages the bleaching effect in the older leaves, due to decomposition of chlorophyll, may be observed in the margins and in the areas between the larger veins. On the upper surfaces of the leaves, however, development of anthocyanin soon follows the disappearance of the normal green, so that when viewed from above the leaves, with the exception of the principal veins, present a bright red appearance. As far as observed the under surfaces of the leaves do not show the red color.

The plots used in studying the minimum magnesium requirements were located at the cooperative tobacco field station at Upper Marlboro, Md., the Connecticut Tobacco Substation at Windsor, Conn., and the North Carolina Tobacco Branch Station at Oxford, N. C. In a limited number of similar tests on Tifton sandy loam soil at the Georgia Coastal Plain Experiment Station at Tifton, Ga., definite symptoms of magnesium deficiency have not been observed, but further work is needed before final conclusions can be drawn. The plan of procedure has been to ascertain the magnesium content of entire plants and of leaves and stalks of plants showing various degrees of magnesium hunger and grown on soils known to be deficient in magnesium, with and without applications of this element to the soil. Since the symptoms of magnesium deficiency first appear on the lower

leaves of the plant, it is a simple matter to find plants showing definite gradations in these symptoms in the leaves at increasing heights on the stalk. The results of the tests are shown in Table 1.

TABLE 1.—*Magnesium content of tobacco leaves manifesting symptoms of magnesium hunger and of comparable leaves free from these symptoms*

[At Upper Marlboro the leaf samples were taken in the third year of the test]

Locality	Plot No.	Leaf sample No.	Magnesium applied each year		Composition of leaf		Visible symptoms of magnesium hunger
			Pounds per acre	Source	Mg	MgO	
					Per cent	*Per cent*	
Upper Marlboro, Md.	13	2	0		0.15	0.25	Well defined.
	13	3	0		.22	.37	None.
	14	5	3	Cottonseed meal	.12	.20	Moderate.
	14	6	3	do	.18	.30	None.
	12	4	120	Dolomitic limestone	.35	.58	Do.
	6–D	13	0		.10	.17	Severe.
	6–B	9	120	Dolomitic limestone	.65	1.08	None.
	3	1–a	110		.08	.13	Severe.
	11	2–a	24	Magnesium sulphate	.42	.70	None.
Windsor, Conn.	7	21	0		.16	.27	Well defined.
	7	22	0		.34	.56	None.
Oxford, N. C.	6	31	0		.20	.33	Moderate.
	7	32	0		.27	.45	None.

The tests at Upper Marlboro, Md., were located on Collington soil, ranging from the sandy-loam to the loamy-sand type. Plots 3, 11, 12, 13, and 14 received as fertilizer 40 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. The phosphoric acid was derived from dicalcic phosphate and the potash from high-grade sulphate. On plot 14 the nitrogen was derived from cottonseed meal, while on the other plots nitrate of soda was used. In addition to the above-mentioned materials, plots 12 and 13 received 1,000 pounds of dolomitic limestone (12 per cent magnesium) and calcitic limestone, respectively, per acre, applied broadcast, and plot 11 received 250 pounds of Epsom salt per acre, applied in the drill. Sample 2 from plot 13 (Table 1) consisted of lower leaves from plants definitely showing magnesium hunger, while sample 3 contained leaves situated immediately above those of sample 2 on the same plants. While there was no evidence of magnesium hunger in sample 3, it is clear that the magnesium supply of these leaves must have been near the minimum requirements for preventing the disease. The same relations apply with respect to samples 5 and 6 from plot 14. Sample 4 from plot 12 was taken from the same position on the plant as samples 2 and 5. Samples 1–a from plot 3 and 2–a from plot 11 likewise are comparable with samples 2 and 5.

Plots 6–b and 6–d (Table 1) received uniformly as fertilizer on an acre basis 30 pounds of nitrogen in the form of dried blood, 48 pounds of phosphoric acid in the form of dicalcic phosphate, and 73 pounds of potash in the form of high-grade muriate. Plot 6-b also received 1,000 pounds of dolomitic limestone per acre. Samples 13 and 9 consisted of lower leaves comparable to similar samples mentioned in the preceding paragraph.

Plot 7 at Windsor, Conn., received as fertilizer on an acre basis 40 pounds of nitrogen derived from dried blood and nitrate of potash,

64 pounds of phosphoric acid derived from dicalcic phosphate, and 80 pounds of potash derived from nitrate of potash. The dicalcic phosphate also supplied 35 pounds of calcium per acre. Three weeks after transplanting, an additional 40 pounds of nitrogen per acre was applied in the form of nitrate of soda. The soil employed in the test is a light sandy loam of the Merrimac series. On one end of the plot, represented by sample 21, decided evidence of magnesium deficiency appeared, while on the other end, represented by sample 22, there was no indication of the disease. The samples consisted of all leaves on the cured plants.

At Oxford, N. C., plots 6 and 7 received uniformly as fertilizer on an acre basis 32 pounds of nitrogen derived from dried blood, 64 pounds of phosphoric acid (and 35 pounds of calcium) derived from dicalcic phosphate, and 36 pounds of potash, the potash being derived from the sulphate in case of plot 6 and from the muriate in case of plot 7. The soil on which the plots were located is the Durham sandy loam. The leaf samples from the plots were composed of all leaves on the cured plants.

The data presented in Table 1 make it possible to determine with a fair degree of accuracy the minimum magnesium content in the leaf of the tobacco plant which will prevent symptoms of magnesium deficiency as evidenced by a premature breaking down of chlorophyll. It appears that the critical point is reached when the content of magnesium falls to approximately 0.2 per cent, which is equivalent to 0.33 per cent magnesia (MgO). When the content falls much below this figure the disease becomes severe. The value of 0.2 per cent magnesium for tobacco showing moderate symptoms at Oxford, N. C., is somewhat too high, since it applies to all the leaves on the plant, whereas only the lower leaves were actually diseased. It is believed that the slightly higher magnesium content in the leaves from plot 7 at Oxford is due to the solvent action of the muriate of potash on the magnesium of the soil. It seems safe to assume that tobacco leaves with a content of not less than 0.25 per cent magnesium or about 0.4 per cent magnesia will not ordinarily show symptoms of deficiency.

According to Willstätter and Stoll *(8)*, the average content of chlorophyll in green leaves is about 0.8 per cent of the total dry weight, and pure chlorophyll contains 2.7 per cent magnesium. On this basis the magnesium contents of the chlorophyll would be less than 0.03 per cent of the dry weight of the leaf. It is evident that in order to prevent breaking down of chlorophyll the total magnesium content of the leaf must be several times the quantity present in the chlorophyll.

In connection with the problem of magnesium deficiency in individual leaves, it is of interest to consider the distribution of this element in the plant as a whole. The results of studies in this direction are shown in Table 2, along with a limited number of observations on other crop plants under similar conditions. The soils employed were the same as for the tests reported in Table 1 except that the soybeans at Oxford, N. C., were grown on Durham coarse sandy loam. The tobacco material was taken mostly from the same plots that were used for the preceding tests, and the fertilizer treatment can be readily ascertained in each case by referring to the plot number in column 2 of Table 1. At Oxford, plot 1 in tobacco received the same fertilizer as plots 6 and 7 except that no potash was applied. At Windsor, Conn., the treatment of plot 1 was the same as that of

plot 7 in Table 1, and plot 5 differed from these only in that 36 pounds of magnesium per acre was applied in the form of Epsom salt. The treatments of corn plots 13 and 12 were identical with those of the tobacco plots of the same numbers. The plots in potatoes and sweetpotatoes received uniformly on an acre basis 40 pounds of nitrogen as dried blood and nitrate of potash, 64 pounds of phosphoric acid and 35 pounds of calcium as dicalcic phosphate, and 80 pounds of potash as nitrate. The soybeans received no fertilizer, but 1,000 pounds of dolomite per acre had been previously applied to plot 2.

TABLE 2.—*Differences in the distribution of magnesium and calcium in the leaves and stalks (and tubers or thickened roots) of tobacco, corn, potatoes, sweetpotatoes, and soybeans when the supply of magnesium in the soil is varied*

Crop and locality	Plot No.	Visible symptoms of magnesium hunger	Percentage of magnesium in—							Percentage of calcium in—					
			Leaves				Stalk	Tubers (or roots)	Whole plant	Leaves			Stalk	Tubers (or roots)	Whole plant
			Lower	Middle	Upper	Average				Lower	Upper	Average			
Tobacco: Upper Marlboro, Md.	6D	Severe	0.10	0.13	0.21	0.13	0.16	----	0.14	----	----	----	----	----	----
	6B	None	.65	.41	.41	.51	.28	----	.43	----	----	----	----	----	----
	13	Well defined	----	----	----	.16	.20	----	.17	----	----	----	----	----	----
	14	Moderate	----	----	----	.18	.24	----	.20	----	----	----	----	----	----
	12	None	----	----	----	.41	.31	----	.38	----	----	----	----	----	----
Oxford, N. C.	6	Moderate	----	----	----	.20	.14	----	.18	----	----	3.46	0.84	----	2.59
	7	None	----	----	----	.27	.11	----	.22	----	----	3.67	.82	----	2.72
	1	do	----	----	----	.37	.16	----	.30	----	----	4.60	1.08	----	3.43
Windsor, Conn.	1	Well defined	----	----	----	.18	.15	----	.17	----	----	----	----	----	----
	5	None	----	----	----	1.24	.27	----	.92	----	----	----	----	----	----
Corn: Upper Marlboro, Md.	13	Well defined	.13	----	.13	.13	.20	----	.18	1.09	0.71	.87	.44	----	.57
	12	None	.35	----	.23	.29	.29	----	.29	.92	.61	.77	.31	----	.44
Potatoes: Upper Marlboro, Md.	1	Doubtful	----	----	----	.22	----	0.12	.18	----	----	1.51	----	0.12	.92
	3	None	----	----	----	.55	----	.13	.34	----	----	1.08	----	.07	.58
Sweetpotatoes: Upper Marlboro, Md.	1	Mild	----	----	----	.40	----	.06	.13	----	----	2.86	----	.20	.71
	3	None	----	----	----	.71	----	.06	.20	----	----	2.59	----	.21	.71
Soybeans: Oxford, N. C.	1	Moderate	----	----	----	----	----	----	.30	----	----	----	----	----	----
	2	None	----	----	----	----	----	----	.59	----	----	----	----	----	----

The results show that when there is definite evidence of magnesium deficiency the upper leaves of the tobacco plant contain considerably more of this element than the lower ones and that the average content of the leaves as a whole does not differ much from that of the stalk. The stalk, however, usually contains slightly more magnesium than the leaves. With a more liberal supply of magnesium available to the plant, the lower leaves contain considerably more than the upper leaves and the leaves as a whole contain decidedly more than the stalk. It appears that symptoms of deficiency may be expected when the content of magnesium in the plant as a whole falls to 0.21 per cent or thereabouts.

Symptoms of magnesium deficiency may occur in young tobacco plants in the seed bed. In one instance in Granville County, N. C., seedlings showing distinct evidence of magnesium hunger were found to contain 0.13 per cent magnesium as compared with 1.48 per cent calcium.

The limited data available indicate that under similar conditions the minimum magnesium requirements of tobacco and corn are about the same so far as shown by the magnesium content of the plant, and about the same relations seem to hold in the distribution between leaf and stalk. With the same conditions as to soil and fertilization which produced magnesium deficiency in tobacco, no distinctive symptoms could be recognized with certainty in potatoes, but in sweetpotatoes a mild though distinct bleaching of the green leaves was seen. The figures shown in Table 2 for average content of magnesium in the leaves of potatoes and sweetpotatoes refer to samples also containing the aerial stems. It is evident that addition of magnesium to the soil increased the content of this element in the potatoes, sweetpotatoes, and soybeans.

PHYSIOLOGICAL AND BIOCHEMICAL RELATIONS IN MAGNESIUM DEFICIENCY

In the earlier work on magnesium-deficiency symptoms (sand drown) in tobacco (*1*), certain biochemical observations were made, but the material employed was taken from plots that were not strictly comparable with respect to magnesium relations, since sulphate of potash was used in one case and muriate of potash in another. It was difficult, therefore, to distinguish with certainty between effects due to magnesium and those due to the sulphate and chlorine ions. Accordingly, additional and more complete studies have been carried out under conditions that were more strictly comparable. Samples of full-grown leaves were taken from a position immediately below the center of the plant, the green weights were ascertained, and the leaf areas were determined by tracing on paper. The leaf tissue was killed by exposure to chloroform vapor, and the material was then quickly dried in the oven. The fertilizer treatments of the plots at Upper Marlboro, Md., on which the plants were grown have been described (p. 147), except that of plot 10, not previously mentioned, which received the same treatment as plot 11 save that the potash and the magnesium were derived from the chlorides instead of the sulphates. The leaves from plots 3 and 13 showed definite symptoms of magnesium hunger. The 1924 samples contained 12 leaves, and the 1925 samples contained 30 leaves. The area, green and dry weights, and water content of the leaves are shown in Table 3.

TABLE 3.—*Effect of magnesium deficiency on green and dry weights, area, and water content of mature tobacco leaves at Upper Marlboro, Md.*

Year	Plot No.	Visible symptoms of magnesium hunger	Total leaf area	Total weight		Moisture content	Weight of leaf per square foot		Weight of water per square foot of leaf
				Green	Dry		Green	Dry	
			Sq. feet	*Gm.*	*Gm.*	*Per cent*	*Gm.*	*Gm.*	*Gm.*
1923	12	None	17.53	418.1	80.3	80.8	23.85	4.583	19.27
	13	Well defined	15.48	418.6	60.6	85.5	27.04	3.915	23.12
1924	3	Severe	22.56	920.6	139.3	84.9	40.82	6.174	34.64
	10	None	30.00	1,103.0	196.5	82.2	36.77	6.550	30.22
	11	do	26.99	969.0	182.1	81.2	35.90	6.746	29.15
	12	do	20.15	706.0	151.7	78.5	35.04	7.528	27.50
	13	Severe	17.96	676.7	119.5	82.3	37.68	6.653	31.01

It is evident that the leaves with symptoms of magnesium deficiency (plots 3 and 13) in all cases had only a slight reduction in total green weight but a decided decrease in total dry weight and, of course, a corresponding increase in water content, and there was considerable reduction in total leaf area. On the basis of equal area of leaf, the green weight appreciably exceeded that of the normal leaf, but the dry weight was considerably less. The marked increase in water content when magnesium was deficient possibly was due to the failure of the stomata to function normally when the chlorophyll pigments had become disorganized. The material on which Table 3 is based was subjected to chemical analysis, and the results are shown in Table 4.

TABLE 4.—*Effect of magnesium deficiency on the composition of mature tobacco leaves at Upper Marlboro, Md.*

PERCENTAGE COMPOSITION OF DRY MATERIAL

Year	Plot No.	Carbohydrates			Organic acids				Protein	Ash		Calcium	Magnesium
		Starch	Reducing sugars (as invert)	Invert sugars (as sucrose)	Citric	Malic	Oxalic	Total		Total	Pure *		
1923	12	27.93	4.12	0.21					8.94				
	13	18.55	4.54	.23					10.06				
1924	3	18.00	7.82	.16	0.76	4.09	1.23	6.08	11.56	14.26	9.70	1.72	0.08
	10	28.74	7.13		.50	1.92	1.05	3.47	11.50	9.67	7.59	1.37	.39
	11	27.53	6.89	.04	.90	3.13	.96	4.99	11.43	10.17	7.61	1.36	.42
	12	29.53	5.38	.11	1.02	2.90	.91	4.83	8.88	9.67	6.96	1.53	.30
	13	20.44	6.12	.11	.80	4.25	1.30	6.35	9.19	15.07	9.52	2.18	.04

WEIGHT IN GRAMS PER SQUARE FOOT OF LEAF AREA, ON DRY BASIS

1923	12	1.22	0.19	0.01					0.44				
	13	.73	.18	.01					.81				
1924	3	1.11	.48		0.05	0.25	0.08	0.38	.69	0.88	0.60	0.11	0.005
	10	1.88	.47		.03	.13	.07	.23	.75	.63	.50	.09	.03
	11	1.94	.48		.06	.22	.07	.35	.81	.72	.54	.10	.03
	12	2.22	.41	.01	.08	.22	.07	.37	.69	.73	.52	.12	.02
	13	1.36	.41	.01	.05	.28	.09	.42	.63	1.00	.63	.15	.003

* Ash free from carbon, carbon dioxide, and silica.

Since the distinctive pathological feature of magnesium deficiency is in the first instance disorganization of the chlorophyll pigments, it is to be expected that assimilation processes would be first affected. This is clearly indicated in the reduction of the starch reserve in the leaves from plots 3 and 13. Marked accumulation of starch is one of the distinctive features of normal maturation in the tobacco leaf, as is shown by the leaves from the other plots. There are no marked differences in sugar content, indicating that diastatic activity has not been greatly disturbed. The protein values are not entirely consistent, but they show that even with moderately severe chlorosis there is no serious breaking down of protein. The apparent increase in ash content is largely a complementary relationship due to loss of carbohydrate, but in part also it involves partial replacement of readily mobile magnesium by the less mobile calcium. In association with the latter relationship there seems to be a slight increase in accumulation of organic acids, probably in the form of calcium salts. The chemical picture as a whole fully agrees with field observations in

showing that, except in very severe cases and in advanced stages, magnesium deficiency involves, from a physiological standpoint, little more than interference with assimilation due to the breaking down of chlorophyll. As a natural result, growth processes are retarded, but the affected tissues may resist final breakdown for a prolonged period.

As has been suggested in a preceding paragraph, the chlorine ion apparently increases the availability of the magnesium in the soil, and in the earlier report on magnesium deficiency (*1*) considerable data were presented bearing on the comparative effects of sulphate and muriate of potash under conditions of restricted magnesium supply. In plots 10 and 11 of the present series, however, the plants received an ample supply of magnesium and the two treatments differed only with respect to chlorine and sulphate ions. The data in Table 3 indicate that, independently of the magnesium supply, the chlorine ion (plot 10) has a definite effect on the water relations and vigor of growth of the tobacco leaf. The physiological effects of chlorine on tobacco will receive more detailed consideration in another publication, and in the present connection it will suffice to point out that this element tends to increase the total green weight of the leaf, due primarily to the increased water content maintained in the tissues. This situation results in a considerable increase in leaf area, and, as noted elsewhere (*6*), it has been observed to exert a decided protection against drought injury in the form of firing or spotting of the leaf.

MAGNESIUM AS A FERTILIZER FOR TOBACCO

In farm practice the occurrence and the severity of the symptoms of magnesium hunger on light-sandy and sandy-loam tobacco soils are greatly affected by weather conditions, particularly the amount and distribution of the rainfall. In comparatively wet seasons evidence of this condition is widespread. It was especially prevalent, for example, in the South Atlantic States and in the Connecticut Valley during the wet summer of 1928. Since attention was first directed to this nutritional disease considerable quantities of dolomitic limestone have been used as a preventive in some sections, and some attention also has been given to the inclusion of magnesium in the tobacco fertilizer. It has been thoroughly demonstrated that application to the soil of comparatively small quantities of suitable forms of magnesium provides an effective preventive of this trouble, but there is as yet little definite information as to the particular types of soil which are likely to be deficient in magnesium. The data presented in the preceding paragraphs make it clear that the particular soils used in these tests are deficient in available magnesium, and it seemed desirable to ascertain the content of these soils in magnesium soluble in hot acid. Analysis was made of representative samples of the soil and subsoil from the plots, or from land adjoining them, at Upper Marlboro, Md., and Windsor, Conn. At Oxford, N. C., the soil samples used for analysis were taken from plots located on Durham coarse sandy loam, and represent the soil on which the data for soybeans (Table 2) were obtained. In this instance the subsoil was not studied. In each instance separate analyses were made of three different samples, but in no case was there any important difference in the results. The average values obtained and the corresponding values for lime content are shown in Table 5.

TABLE 5.—*Magnesium and calcium content (water-free basis) of soils used in plot tests to determine the minimum magnesium requirements of tobacco*

Locality	Type of soil	Magnesium content	Calcium content
		Per cent	*Per cent*
Windsor, Conn.	Merrimac sandy loam, soil	0.24	0.32
	Merrimac sandy loam, subsoil	.27	.35
Upper Marlboro, Md.*	Collington loamy sand, soil	.10	.07
	Collington loamy sand, subsoil	.21	.07
Oxford, N. C.	Durham coarse sandy loam, soil	.024	.09

* Separate samples of soil were used for the magnesium and the calcium determinations.

These results are especially interesting as indicating the wide range in content of magnesium in soils under which deficiency symptoms may occur. These symptoms have been but little less prominent on the Windsor than on the Oxford soil, although the former contains 10 times as much magnesium soluble in strong acid as the latter. On the basis of data given in Tables 1 and 2, a tobacco crop of about 1,200 pounds of dry leaf and 600 pounds of stalks was able to obtain from the Windsor soil only about 3.3 pounds of magnesium, despite the fact that the potential supply in the first 7 inches of the soil is estimated to have been about 6,000 pounds. Obviously, the availability of the magnesium is a very important factor. In Europe, Joulie has often been quoted (*3*) as holding that productive soils should contain a minimum of 0.18 per cent magnesium (0.30 MgO), but it is apparent from the results on the Windsor soil that a content of 0.24 per cent is not always adequate, at least in the case of tobacco. On the other hand, it is undoubtedly true that magnesium hunger in tobacco will not always occur on soils containing considerably smaller quantities of magnesium. The relative availability of the magnesium and the prevailing weather conditions seem to be factors of major importance.

Numerous chemical analyses of soil types commonly employed in growing tobacco have been reported, but many of these do not include determination of the magnesium content. So far as this value has been given, a large proportion of the figures for the sandy, sandy-loam, and fine sandy-loam types of soil commonly used in growing the flue-cured, Maryland, and cigar wrapper and binder leaf types of tobacco run from 0.1 per cent down to mere traces of magnesium. It should not be surprising that symptoms of magnesium hunger are frequently seen on many of these soils. The heavier loams and silt loams on which dark air-cured and fire-cured, Burley, and cigar-filler and binder types of tobacco are grown apparently contain, as a rule, an ample supply of magnesium for the requirements of the crop. A limited number of experiments conducted on these soils seem to support the analytical data on this point.

In contrast with the variable and uncertain availability of the natural magnesium content of the soil stands the effectiveness and certainty with which applications of soluble salts of magnesium and dolomitic limestone to the soil prevent any evidence of magnesium hunger in the crop. The effect of adding magnesium sulphate to the soil in increasing the magnesium content of the tobacco crop is

well shown in the experiments at Windsor, Conn., some features of which have been given in the preceding tables. The results of these experiments are presented in part in Table 6.

TABLE 6.—*Effect of adding magnesium sulphate to the soil on the content of magnesium in the tobacco crop at Windsor, Conn.*

Plot No.	Magnesium applied per acre	Magnesium content of—			Magnesium [a] removed in crop
		Leaf	Stalk	Whole plant	
	Pounds	*Per cent*	*Per cent*	*Per cent*	*Pounds*
1	0	0.18	0.15	0.17	3.3
3	18	.84	.22	.63	14.1
5	36	1.24	.27	.92	19.9

[a] Data based on analyses here shown and actual plot yields of leaf.

The addition of only 18 pounds of water-soluble magnesium (30 pounds MgO) per acre (in the drill) increased the magnesium content of the leaf four times, and the addition of 36 pounds raised the content to seven times that of the leaf in the control planting. The effect on the magnesium content of the stalk was relatively small. The crop as a whole was able to recover much more than half of the 18 pounds of magnesium added to the soil and about half of the 36 pounds applied. In experiments elsewhere the recovery of soluble magnesium applied as fertilizer, although not always so large, has been quite satisfactory without exception. Over a period of several years the additions of magnesium in the Windsor tests have given moderate increases in yield, but the greater effect has been on the quality of the cured leaf.[3] So far as known at present 18 pounds of water-soluble magnesium per acre applied as a fertilizer is ample for preventing symptoms of magnesium hunger or sand drown, and in most cases half of this quantity probably will be effective. It may well be, of course, that larger quantities of magnesium are needed for the production of maximum yield and quality in tobacco than are required to suppress deficiency symptoms. Some data on this point are presented on page 163. Sulphate of potash-magnesia, which contains 5 to 7 per cent magnesium, and sulphate of magnesia appear to be effective sources of water-soluble magnesium for tobacco.

Dolomitic limestone has given uniformly good results as a remedy for magnesium hunger when applied either broadcast or in the drill. Larger quantities of magnesium in this form are required, of course, than when water-soluble salts are used. The detailed results of extensive experiments over a period of years at Oxford, N. C., on the value of dolomitic limestone for the tobacco crop have been reported elsewhere (*5*, *6*). These results show highly profitable returns, and results from somewhat similar tests at Upper Marlboro, Md., which have not yet been published, also show decided benefit from the use of this material. In these experiments the usual practice has been to apply 1,000 pounds of the limestone per acre in the drill or a ton broadcast, although in some instances 500 pounds in the drill has

[3] Credit is due H. F. Murwin, agent, of the Office of Tobacco and Plant Nutrition, for having secured data on yield and quality of the crop under the several treatments. For further details of these experiments see Murwin (*7*).

proved to be sufficient. In some experiments the limestone has been applied each year, while in others it has been applied broadcast once every three years. The results as a whole seem to indicate that 100 to 200 pounds of actual magnesium per acre in the form of limestone will effectively control deficiency symptoms. In the tests at Oxford, N. C., a ton of dolomitic limestone (12 per cent magnesium) per acre applied broadcast once in every three years has been effective.

Experience has shown that the magnesium contained in organic materials of plant and animal origin, such as cottonseed meal, tobacco stems, and barnyard manure, may effectively prevent deficiency symptoms in tobacco when sufficient quantities of the materials are applied to the soil. This is probably one reason why cottonseed meal, which usually contains about 0.6 per cent magnesium, has been so popular in some sections as a constituent of the tobacco fertilizer. Castor pomace also contains about 0.5 per cent magnesium. The data in Tables 1, 2, and 4 showing the magnesium content of tobacco as affected by the available supply in the soil illustrate the variation to be expected in the content of magnesium in crop residues grown on various soils and with various systems of fertilizing. Because of these variations in soil and in fertilizer practice precise data as to the fertilizer value of crop residues with respect to magnesium content can not be given. It appears that the straws of wheat, oats, rye, and barley, as well as grass hay, usually contain 0.1 to 0.15 per cent magnesium, while corn stover ordinarily ranges from 0.15 to 0.3 per cent, and clover hay from 0.3 to 0.5 per cent. For barnyard manure with 75 per cent water, a large number of analyses indicate an average magnesium content of 0.09 to 0.12 per cent, as compared with 0.35 to 0.65 per cent calcium, 0.4 to 0.6 per cent potash, and 0.15 to 0.35 per cent phosphoric acid. The few data available for products of animal origin indicate a magnesium content of 0.15 to 0.3 per cent in tankage and dried ground fish, while dried blood seems to be almost free from magnesium. Wood ashes, which have been extensively used as a fertilizer for tobacco in some sections, usually contain on a dry basis 2 to 3 per cent magnesium. In the fertilizer experiments at Oxford, N. C., basic slag and bone meal have tended to prevent symptoms of magnesium hunger in tobacco, but the quantity of magnesium in these materials was not determined. Rock phosphate and apatite, and therefore superphosphate, sometimes contain small percentages of magnesium.

SYMPTOMS OF CALCIUM DEFICIENCY IN TOBACCO

As previously stated, when calcium is not present in sufficient quantity in the culture medium magnesium may be highly toxic to plants. For this reason it is somewhat difficult to distinguish satisfactorily between symptoms of magnesium toxicity and symptoms of actual calcium deficiency. Since the present discussion of the calcium requirements of tobacco is only preliminary, no attempt will be made to review the literature on the functions of this element in plant nutrition and on the deficiency symptoms developed in water cultures when it is not present in sufficient quantity. The main purpose is to direct attention to the fact that definite calcium-deficiency symptoms may develop in tobacco grown on certain soils unless this element is supplied in proper quantity in the fertilizer or by other means. Evidence

of this fact was first brought to light in connection with a series of plot tests with nitrogen carried out on the Collington loamy-sand type of soil at Upper Marlboro, Md., in collaboration with D. E. Brown, of the Office of Tobacco and Plant Nutrition, the detailed results of which will be presented elsewhere. In these tests provision was made for including commercial Ammo-phos containing 11.5 per cent nitrogen and 45 per cent phosphoric acid. This material was used at two rates in conjunction with sulphate of potash, to supply 20 and 40 pounds nitrogen, 80 and 160 pounds phosphoric acid, and 40 pounds potash per acre. The yields obtained as compared with those produced by equivalent quantities of ammonium sulphate and urea were very poor, and, in fact, were but little better than where no nitrogen at all was used. Evidences of magnesium deficiency having appeared in certain other plots in this series of nitrogen tests, after two years dolomitic limestone containing 12 per cent magnesium was applied uniformly on all plots at the rate of a ton per acre. For a period of six years since the addition of the limestone the Ammo-phos has given substantially as good yields as the other forms of nitrogen.

Subsequently, on the same soil type a test was begun with a mixture of almost pure ammonium phosphate and nitrate of potash furnishing 40 pounds nitrogen, 60 pounds phosphoric acid, and 90 pounds potash per acre (plot A). This mixture was compared with a fertilizer supplying the same quantities of nitrogen, phosphoric acid, and potash in which the nitrogen was derived in equal proportions from cottonseed meal and nitrate of soda, the phosphoric acid from superphosphate, and the potash from the sulphate (plot B). As to the outcome of the test, it will suffice to state that after the first year very poor results were obtained with the ammonium phosphate-nitrate of potash mixture. A large proportion of the plants, in fact, were but little larger at the end of the growing season than when first set in the field. Unfavorable results also have been obtained with this mixture on other sandy and sandy loam soils. Here, as in the Ammo-phos test, however, the addition of dolomitic limestone to the soil largely, if not entirely, corrected the trouble.

In the above-mentioned tests the outstanding feature of the poor results has been the failure of the plants to grow, although distinct evidence of chlorosis in the leaves also has been observed. Additional field experiments, involving the use of pure chemicals, together with pot tests, were next undertaken to reach an explanation of the favorable action of dolomitic limestone in bringing about normal response by the plant to Ammo-phos and the combination of ammonium phosphate and nitrate of potash. In a plot test with a combination of ammonium nitrate, monoammonium phosphate and ammonium sulphate (plot 1) furnishing 40 pounds of nitrogen and 64 pounds of phosphoric acid per acre but no potash, magnesium, or calcium, there was poor growth in the tobacco the first year and almost no growth thereafter.

The most prominent disease symptoms were those of potash hunger. With an addition of 80 pounds of potash per acre in the form of muriate (plot 2) the symptoms of potash hunger disappeared, but still the plants made very little growth, as is shown in Figure 1. When 12 pounds of magnesium per acre in the form of sulphate was added to the fertilizer mixture (plot 3) there was marked improvement in growth of the plants, as is seen in Figure 2. In this case, however, the upper

leaves of the plants showed striking and characteristic abnormalities in development. These abnormalities are well brought out in Figures 3 and 4, which show an individual plant and an individual leaf of a

FIGURE 1.—Plot of tobacco (four rows) which received as fertilizer a mixture of ammonium nitrate, ammonium sulphate, monoammonium phosphate, and muriate of potash in proportions to supply 40 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. The photograph was made near the end of the growing season. Similar results have been obtained with ammonium phosphate and nitrate of potash. (Compare with Figures 2 and 3.) Scale in feet

plant, respectively, from this plot. The tips and margins of the upper leaves failed to develop normally, and some of the leaves appear as if sections of the laminae had been cut away. There is some mottling,

FIGURE 2.—Plot of tobacco (four rows) which received the same fertilizer as that shown in Figure 1 except 12 pounds of magnesium per acre was added in the form of sulphate. Growth was greatly stimulated, but the upper leaves showed striking abnormalities in development. Scale in feet

but this is not a dominant feature. The lower leaves are normal. This trouble shows little or no resemblance to either potash hunger or magnesium hunger. Similar results were obtained when the ammonium

FIGURE 3.—Individual plant from the plot shown in Figure 2, illustrating abnormalities in leaf development which resulted from a deficient supply of calcium

FIGURE 4.—Individual leaf from plot shown in Figure 2, illustrating in greater detail abnormalities in development associated with an inadequate supply of calcium. As shown in Figure 6, the addition of 36 pounds of calcium per acre fully corrected this pathological condition

phosphate was replaced by monopotassium phosphate, with proper reduction in the quantity of muriate of potash applied, so as to keep the total quantity of potash constant (plot 4).

The next step was to supply calcium as a nutrient. This was done by replacing the ammonium phosphate with an equivalent supply of dicalcic phosphate, thus supplying about 36 pounds of calcium per acre. The ammonium nitrate also was replaced with nitrate of soda for a purpose not bearing on the present problem (plot 5). Adding the calcium to the fertilizer mixture had the effect of producing an entirely normal crop of tobacco, as is shown in Figure 5. That the nitrate of soda was not a factor in the improvement obtained is shown by the fact that in an adjoining plot, identical in treatment with plot 3 except that nitrate of soda was substituted for the

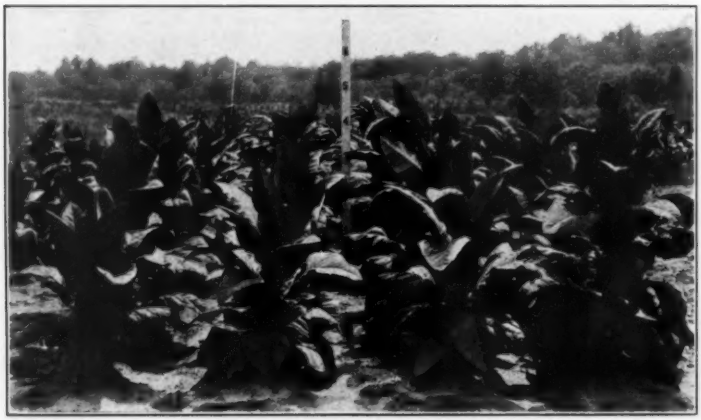

FIGURE 5.—Plot of tobacco which received the same fertilizer as that shown in Figure 2 except that the ammonium phosphate was replaced by dicalcic phosphate and nitrate of soda was substituted for ammonium nitrate. It will be seen that this treatment produced an excellent crop. In numerous supplementary tests ammonium nitrate has given excellent results, and the great improvement in the crop over that in Figure 2 is to be attributed to the calcium supplied in the phosphate. Scale in feet

ammonium nitrate, the tobacco showed the same abnormalities as that in plot 3. Thus, it appears that on the soil in question both magnesium and calcium are essential constituents of the complete fertilizer for tobacco. It should be stated here that this soil is very nearly neutral in reaction, the pH value ranging from 6.5 to 6.8. The interesting question arises whether in these plot tests the striking abnormalities in leaf development which appeared when calcium was not included in the fertilizer were really due specifically to calcium hunger as such or are to be regarded as manifestations of magnesium toxicity in the absence of calcium. These tests in themselves do not permit of a definite answer, but they do show that if magnesium toxicity was involved potash was wholly ineffective as an antagonistic agent.

In this connection it should be recalled that in the ammonium phosphate-nitrate of potash test, where neither magnesium nor calcium was added to the soil, no clearly defined symptoms of calcium deficiency could be seen. This fact suggests that the observed effects

are due, at least in part, to magnesium toxicity. In any event a small quantity of calcium in the fertilizer has proved to be an effective remedy for the trouble. These results in the field were confirmed in pot tests with the same soil. In order to accentuate the effects, the soil was occasionally leached with an excess of dilute nutrient solutions supplying nitrogen, phosphorus, potassium, magnesium, and sulphur, with and without calcium. In the absence of calcium the growing point and upper leaves of the plant invariably showed characteristic pathological effects similar to those seen in the field tests, and only the lower leaves were able to develop normally. The growing point is affected to such an extent that commonly the plant is unable to flower. These tests have shown that magnesium hunger affects primarily the lower leaves of the plant, whereas calcium hunger affects most seriously the upper leaves and embryonic tissues of the growing point. Since the soil in question is deficient in both magnesium and calcium, it is easy to see that anything like normal growth of the plant is impossible when fertilizers are used which supply only nitrogen, phosphoric acid, and potash. When only one of the deficient elements, magnesium or calcium, is added to the fertilizer, growth of the plant is markedly stimulated, but there results a correspondingly marked accentuation of the pathological deficiency symptoms of the element which has not been supplied.

CALCIUM REQUIREMENTS OF TOBACCO

With evidence in hand that under practical field conditions calcium deficiency may occur in some tobacco soils, it was of interest to obtain information as to the minimum content of calcium in the plant which will prevent deficiency symptoms. With this in view, samples of upper and lower leaves of plants were secured from some of the plots at Upper Marlboro, Md., which have been discussed in preceding paragraphs, and the content of magnesium and calcium in the samples was determined. Details of the plot treatments have already been given (p. 156 and 160). In plot A, involving the use of ammonium phosphate and nitrate of potash, the growth of plants on one end (designated A–1) was considerably poorer than that on the other end (designated A–2), and separate samples were taken from the two ends of the plot. The results of the analyses are shown in Table 7.

TABLE 7.—*Calcium and magnesium contents of tobacco showing calcium-deficiency symptoms, and of comparable material free from such symptoms, grown at Upper Marlboro, Md.*

Plot No.	Calcium per acre supplied in fertilizer	Position of leaves	Calcium-deficiency symptoms	Calcium content	Magnesium content
	Pounds			*Per cent*	*Per cent*
3	0	Upper	Pronounced	1.00	0.57
3	0	Lower	Not pronounced	1.00	.72
5	35	Upper	None	1.93	.46
5	35	Lower	do	2.29	.57
A–1	0	Upper	Doubtful	.94	.26
A–1	0	Lower	do	1.30	.26
A–2	0	Upper	None	1.33	.32
A–2	0	Lower	do	1.90	.26
B	50	Upper	do	1.87	.31
B	50	Lower	do	2.43	.23

On plot A no magnesium was supplied in the fertilizer, and on plot B only an insignificant quantity was carried in cottonseed meal used in the fertilizer. In these cases it is apparent that the magnesium content in the lower leaves of the plant is dangerously near the point at which magnesium-deficiency symptoms make their appearance. The magnesium content of the upper leaves equals or exceeds that of the lower ones. The distribution of calcium between upper and lower leaves is decidedly in favor of the latter. This relationship in distribution is in line with the fact that magnesium deficiency first becomes apparent in the lower leaves, whereas ordinarily calcium deficiency is first seen in the upper ones. The marked increase in calcium content in the leaves resulting from addition to the soil of 50 pounds of calcium per acre, seen in comparing treatments B and A, without material change in magnesium content, confirms the conclusion that calcium deficiency was the major cause of poor growth in plot A–1. Furthermore, it appears that with a low magnesium content a calcium content of 1.33 per cent in the upper leaves was sufficient to admit of good growth free from deficiency symptoms, whereas a content of 0.94 per cent was clearly inadequate. In treatments 3 and 5, 12 pounds of magnesium per acre in the form of sulphate was supplied in the fertilizer, and the magnesium content of the tobacco leaves was well above the minimum requirements. In treatment 3, in which the soil received no calcium, the content of this element in the leaves (1 per cent) was sufficient to admit of fair growth, but with development of marked deficiency symptoms. As compared with results on plot A–1, the additional magnesium stimulated growth but accentuated the calcium-deficiency symptoms. On plot 5, as on plot B, a small quantity of calcium in the fertilizer doubled the calcium content of the tobacco leaves and resulted in excellent growth and freedom from deficiency symptoms.

The limited data here presented do not justify sweeping conclusions, but they do show that under the conditions of the tests the minimum calcium requirement of tobacco, as measured by calcium content of the upper leaves, lies between 1.1 and 1.5 per cent (1.5 to 2.1 per cent CaO). The minimum calcium requirement, therefore, is about four or five times the minimum magnesium requirement. It is quite possible that with increased content of magnesium in the leaves the calcium requirement would be materially increased. In the light of published data for other crops it would seem that the calcium requirements of tobacco are relatively high. The data from plots 3 and 5 and those in Table 2 relating to corn and potatoes seem to indicate that magnesium may replace calcium to some extent in the plant when the calcium supply is deficient, but with decidedly harmful results, at least in the case of tobacco.

It seemed desirable to ascertain the potential calcium supply of the soil used in the above-described tests and of the other soils which have proved to be deficient in magnesium. The results are shown in Table 5. It is seen that the Collington loamy sand, which is unable to furnish sufficient calcium for the needs of the crop, contains in both the topsoil and the subsoil 0.07 per cent soluble in hot hydrochloric acid. The calcium content of the Merrimac sandy loam is somewhat greater than the magnesium content. The Durham coarse sandy loam is low in calcium, although the content is appreciably higher than that of magnesium. Whether the Merrimac and

Durham types will produce calcium-deficiency symptoms in tobacco has not been determined. Available data on the calcium content of tobacco soils are somewhat more extensive than those on the magnesium content. Where comparison is possible the average calcium content exceeds considerably the average magnesium content of the sandy and sandy-loam tobacco soils. It is also true, however, that the calcium requirements of the crop considerably exceed the magnesium requirements. Moreover, a comparatively large proportion of the analyses of these soils show a calcium content of less than 0.1 per cent, suggesting that the available supply may not be sufficient for normal crop production. A considerable amount of experimentation will be required before a definite conclusion can be reached as to the extent to which deficiency symptoms and effects are to be expected on these soils when fertilizers free from calcium are used.

BALANCING MAGNESIUM AND CALCIUM IN TOBACCO FERTILIZER

In undertaking to supply magnesium and calcium as nutrients for the tobacco crop in soils found to be deficient in these elements, certain important considerations must be kept in mind, although from a practical standpoint the problems involved are relatively simple. The two most important considerations are: (1) Magnesium may be toxic to the plant unless a sufficient supply of calcium is present; (2) the soil reaction is of considerable significance from the standpoint of disease. The data presented herein, together with the results of pot tests which will not be here considered in detail, show that magnesium may be decidedly toxic even when applied to the soil in the form of finely divided carbonate. Water-soluble magnesium is readily absorbed by the plant, and it has been shown in preceding paragraphs that only 12 pounds per acre applied as sulphate resulted in markedly abnormal growth in tobacco when calcium was deficient in the soil. In the same tests, however, a small quantity of calcium in the form of phosphate was quite effective in preventing this abnormality. In this connection it will be recalled that ordinary superphosphate (acid phosphate) may contain 18 per cent or more of calcium, partly as phosphate and partly as sulphate. Where this material is used at ordinary rates, it would be expected to supply sufficient calcium to prevent deficiency symptoms and also counteract magnesium toxicity unless the magnesia is present in large quantities. An experiment on Durham coarse sandy loam at the Oxford (N. C.) Branch Tobacco Station supports this conclusion. In a test begun in 1923 a fertilizer mixture composed of dried blood, superphosphate, and sulphate of potash and furnishing 32 pounds of nitrogen, 64 pounds of phosphoric acid, and 36 pounds of potash per acre was applied uniformly to all plots. Magnesium was applied in the form of sulphate at rates of 0, 12, 24, 36, and 48 pounds per acre. The results are summarized in Table 8.

The 1925 crop was destroyed by fire, so the results in Table 8 cover a period of only four years. The soil evidently contained a fair reserve of magnesium, for there have never been any clearly defined deficiency symptoms on the control plots. Nevertheless, it is apparent that up to 36 pounds of magnesium per acre has given appreciable increases in yield and value, while best results have been obtained with 24 pounds per acre. Even the 48-pound rate has only

slightly decreased the yield, and there have been no definite symptoms of toxicity. The calcium contained in 400 pounds of superphosphate apparently has prevented any toxic effects from the application of as much as 36 pounds of water-soluble magnesium.

TABLE 8.—*Effect of water-soluble magnesium when used in conjunction with superphosphate on yield and value of the tobacco crop at Oxford, N. C., during four different years*

Magnesium per acre applied in fertilizer (pounds)	Yields of tobacco (pounds per acre)					Value of crop (dollars per acre)				
	1923	1924	1926	1927	Average	1923	1924	1926	1927	Average
0	1,308	817	924	811	965	414	204	284	267	292
12	1,238	870	1,017	880	1,001	382	223	309	287	300
24	1,348	855	1,008	997	1,052	434	218	330	322	326
36	1,210	785	984	1,007	997	376	200	316	335	307
48	1,120	738	805	1,023	922	355	182	266	342	286

The experiments with less readily soluble forms of magnesium have been practically limited to the use of dolomitic limestone. A series of comparative tests with dolomitic limestone, calcitic limestone, and no lime—the limestones being applied in the drill each year at the rate of 1,000 pounds per acre and used in conjunction with different sources of potash—has been in progress since 1921. The details of these experiments have been reported in part elsewhere (*5*, *6*). The plots receive as fertilizer a mixture of dried blood, superphosphate, and potash salts supplying 32 pounds of nitrogen, 64 pounds of phosphoric acid, and 36 pounds of potash per acre. The potash salts used are sulphate, muriate, sulphate of potash-magnesia, and kainite. Where muriate and sulphate of potash are used without calcium and with calcite, magnesium-deficiency symptoms have been severe. The magnesium contained in the other potash salts has prevented the deficiency symptoms. All plots receiving dolomitic limestone have remained free from the symptoms of magnesium deficiency. More recently the calcite plots are falling behind the unlimed plots in yield, suggesting that the calcite has the effect of depressing the solubility of the magnesium in the soil, in accordance with the findings of MacIntire and his associates (*2*). The point of special interest in the present connection is that after an annual application of 1,000 pounds per acre of dolomitic limestone for a period of eight years there is as yet no evidence of magnesium toxicity. As a matter of fact, recent pot tests indicate that dolomitic limestone may be used successfully as a medium for growing tobacco plants by simply applying a suitable nutrient solution. With an excess of calcium over the magnesium in dolomitic and magnesian limestones there is, perhaps, no reason for expecting toxic effects from the free use of such materials.

The present discussion centers around magnesium and calcium as plant nutrients and is distinct from the question of liming as such. The reaction of the soil, however, is of practical importance from the standpoint of both disease and nutrition conditions of the plant. The tobacco plant will tolerate a moderately acid soil reaction, but it occasionally happens that the acidity is excessive. In a series of fertilizer tests which have been in progress at the Oxford Tobacco Branch Station since 1913 there is a treatment consisting of ammo-

nium sulphate, superphosphate, and sulphate of potash in quantities to furnish 25 pounds of nitrogen, 64 pounds of phosphoric acid, and 80 pounds of potash per acre. Continued use of this mixture apparently has depleted the supply of alkaline earths in the soil and increased the acidity to the point where, in addition to marked symp-

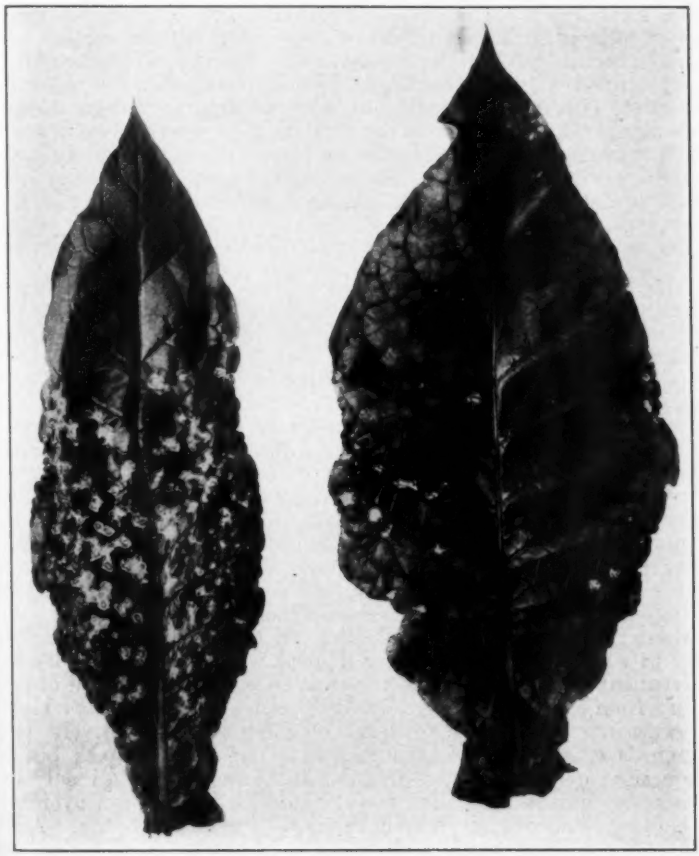

FIGURE 6.—Tobacco leaves from a plot fertilized for a period of years with ammonium sulphate, superphosphate, and sulphate of potash, without liming. The acidity of the soil has increased to the point where severe symptoms of manganese toxicity develop in the upper leaves of the plant, as here shown. The lower leaves of the plant also develop magnesium-deficiency symptoms. Calcitic limestone removes the manganese toxicity, while dolomitic limestone overcomes both troubles

toms of magnesium deficiency, there are in the upper leaves of the plant definite disease symptoms of a different character, probably due to manganese toxicity. These latter symptoms, which appear initially as reddish or brownish cankers on the upper leaves, are accentuated when a nutrient solution devoid of calcium is applied to pot cultures with soil from the plot in question. Leaves showing two stages of this effect are seen in Figure 6. In this case applications of dolo-

mitic limestone have given highly beneficial and profitable results, the average value of the crop for a period of five years having been increased from $78 to $174 per acre (*5*, *6*). Where magnesium is not deficient, presumably any of the ordinary forms of lime may be used to correct excessive soil acidity.

On the other hand, it has been shown at the Connecticut Agricultural Experiment Station (*4*) that serious injury from the Thielavia root-rot disease of tobacco is likely to result if the soil acidity is reduced much below the point represented by the pH value 5.6, at least under the local conditions. Hence, the quantity of dolomitic limestone that may be safely or advantageously used on tobacco soils is not limited primarily by likelihood of toxic effects from its content of magnesium, but rather by the possibility of unduly lowering the acidity of the soil. Moreover, the reaction of the soil is a factor in determining the best form in which magnesium and calcium may be supplied.

CONCLUSION

Magnesium deficiency and calcium deficiency produce in the tobacco plant specific symptoms which are easily recognized under field conditions. Experimentation and observation have demonstrated that on a large proportion of the light-sandy and sandy-loam tobacco soils magnesium hunger is likely to develop in tobacco and even in other crops unless this element is supplied as a fertilizer. It now appears also that at least some of these soils require the addition of calcium as a fertilizer to prevent symptoms of calcium hunger and avoid serious crop injury. As long as farm manures and the old type of fertilizer, consisting largely of organic materials of plant and animal origin, were employed on these soils the necessary quantities of magnesium and calcium were usually supplied and no deficiency symptoms could be seen in the crop. With increased use of more highly concentrated inorganic and organic forms of nitrogen and potash in the fertilizer, magnesium deficiency has become an important factor in tobacco production on the soils in question. So long as superphosphate or other lime phosphate remains a leading constituent of the fertilizer there seems to be little likelihood of crop injury from calcium hunger. However, attempts to use very highly concentrated fertilizers consisting of mixtures of relatively pure chemicals supplying nitrogen, phosphoric acid, and potash but no magnesium or calcium have in some instances given decidedly unsatisfactory results. In such cases the difficulty has been overcome by adding small quantities of both magnesium and calcium. Addition of magnesium alone under these circumstances may result in evidences of magnesium toxicity. Potash apparently does not function effectively in preventing this toxicity.

SUMMARY

In this paper information in some detail is given concerning the symptoms and effects of magnesium deficiency in tobacco, the magnesium requirements of the crop, including comparison with certain other crop plants, and suitable means of supplying these requirements. Preliminary data also are given on symptoms of calcium deficiency in tobacco in close association with magnesium toxicity and the cal-

cium requirements of the crop, these data being derived largely from field experiments with highly concentrated fertilizers supplying nitrogen, phosphoric acid, and potash but no magnesium or calcium. Sharp distinction is made between supplying the magnesium and calcium requirements of the crop and the problem of liming the soil.

Under field conditions the outstanding feature of magnesium deficiency symptoms in tobacco (popularly known as sand drown) is a breaking down of both the green and the yellow chlorophyll pigments, which begins in the lower leaves of the plant and at the tips of the affected leaves. The bleached appearance of the leaves is distinctive. Magnesium hunger differs from potassium hunger in that the leaf surface ordinarily remains smooth, there is no downward curvature of the tips and margins, and specking or spotting resulting from localized breaking down of the tissue rarely occurs.

In magnesium deficiency the size of the leaves is somewhat reduced, while per unit area there is a decided decrease in dry matter and a well-defined increase in water content. There is a decrease in the carbohydrate content of the leaf. In the cured leaf the effects are most evident in the flue-cured type. Affected parts of the leaf are abnormally thin and nonelastic, have a papery texture, and usually show a dull, lusterless, light-brown color.

Analysis of the plant indicates that a minimum content of about 0.25 per cent magnesium or 0.4 per cent magnesia (MgO) in the leaf is required to prevent deficiency symptoms. With an available supply of magnesium no greater than the minimum requirements of the plant, the content of magnesium is somewhat higher in the upper than in the lower leaves, while the content in the stalk is about the same as the average for all leaves on the plant. With a more liberal supply of magnesium the highest percentage is found in the lower leaves and the content in the leaves as a whole greatly exceeds that in the stalk. On the basis of the above figures, the minimum requirements for a crop of 1,000 pounds leaf and 500 pounds stalks would be about 2.5 and 1.25 pounds, respectively, of magnesium (4 and 2 pounds MgO), or a total of about 6 pounds magnesia (MgO).

Experimentation and observation show that magnesium-deficiency symptoms are rather widely prevalent on some of the most important sandy and sandy-loam tobacco soils, especially in seasons of comparatively heavy rainfall. Chemical analysis shows that some of the soils are very low in magnesium, and, moreover, severe deficiency symptoms have been observed even on soils containing up to 0.24 per cent magnesium (0.4 MgO) soluble in strong acid.

Experience indicates that when applied in the drill 12 to 18 pounds of water-soluble magnesium (20 to 30 pounds MgO) per acre, and often less, will effectively prevent symptoms of magnesium deficiency. It has been shown also that 500 to 1,000 pounds per acre of dolomitic limestone applied either in the drill or broadcast will effectively control this trouble. Certain fertilizer materials, barnyard manure, and various crop residues contain beneficial quantities of magnesium.

Preliminary studies have shown that when highly concentrated fertilizers that contain no magnesium or calcium, such as a mixture of ammonium phosphate and nitrate of potash, are used on some of the sandy and sandy-loam soils the tobacco crop makes very poor growth. Addition of water-soluble magnesium to such a fertilizer

greatly stimulates growth, but a pathological condition develops in the upper portion of the plant and the leaves show striking abnormalities. By adding calcium along with the magnesium a normal crop is obtained.

It is difficult to distinguish clearly between the effects of magnesium toxicity and those due to calcium deficiency as such, but in any case an inadequate supply of calcium results in aborted leaf development in which there are large indentations in the margins and the tips are wanting. Preliminary studies indicate that the minimum content of calcium in the leaf required to prevent deficiency symptoms is in excess of 1 per cent, or some four or five times the magnesium requirement.

In the absence of an adequate supply of calcium, toxic effects may be expected from even comparatively small quantities of soluble magnesium, but the calcium contained in 400 pounds of superphosphate has effectively prevented toxic effects from 36 pounds of water-soluble magnesium applied as fertilizer. Dolomitic limestone, because of its calcium content, may be used freely without fear of inducing magnesium toxicity, the consideration limiting the quantity that may be advantageously used being a possible danger of unduly lowering soil acidity and thereby favoring the development of root diseases.

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FIELD TESTS WITH TREATED SEED CORN[1]

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INTRODUCTION

Much interest has developed in recent years concerning possible benefits to be derived from the treatment of seed corn with certain chemical compounds before planting. Although importance was originally attached to the question of growth stimulation, seed treatment has come to be regarded as chiefly a problem of disinfection of the seed to kill disease-producing organisms on the surface and to protect the planted seed from organisms in the soil which cause decay.

Following the intensive studies made during the last decade by a number of investigators, including Hoffer and Holbert (*5*, *7*),[2] Durrell (*3*), Holbert, Burlison, Koehler, Woodworth, and Dungan (*6*), and others, concerning the seedling diseases of corn, it was but natural that investigation should turn to the possible control of these diseases by the use of fungicides. Such investigations have been fruitful, and it has been shown by Holbert, Reddy, and Koehler (*8*), Reddy, Holbert, and Erwin (*13*), Melhus, Reddy, Raleigh, and Burnett (*12*), and Clayton (*2*), that a number of seed-borne seedling-disease organisms are more or less subject to control by certain mercuric disinfectants. These organisms, chief among which are *Diplodia zeae*, *Gibberella saubinetii*, and *Basisporium gallarum*, also belong to the group causing dry rots of the ear. All of these have rather similar life histories and respond similarly to seed treatments. These organisms are all found to some extent in Nebraska, Diplodia being the most prevalent.

Much confusion exists among corn growers as to just what kind of corn diseases may be influenced by seed treatment and to what extent the quality of the crop may be benefited. Many have mistakenly gained the impression that seed treatment should prevent rotten, moldy, smutty, or otherwise diseased ears in the crop. In some years considerable losses are experienced from these ear rots. Especially is this true in seasons of slow growth and late maturity. Varieties of corn that are poorly adapted by virtue of being too late in ripening are also more subject to the development of ear rots late in the season than are well-adapted sorts.

The dry-rot disease organisms which may be expected to respond to seed treatment may each cause diseases of two kinds—seedling blights in the very early stages of plant growth, and stalk and ear rots in the more advanced stages. These rots have never been shown to be related to the seedling blights, and the two kinds of diseases may be regarded as independent, even though the same causal organism is involved.

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[2] Reference is made by number (italic) to "Literature Cited," p. 189.

Stalks, ear shanks, cobs, and grain may become infected during growth by these organisms, the spores of which are usually present in corn-crop residues of previous years. The amount of infection and severity of development of these stalk and ear rots vary both regionally and seasonally and to some extent with cultural practices and varietal type grown, but they are not influenced by the presence of organisms in the seed planted. Therefore no seed treatment need be expected to influence the percentage of diseased or rotted ears in the crop grown.

If seed from ears which have become infected as indicated above is planted, the other expression of disease, namely, seedling blight, may result from the viable seed so infected. Under conditions favorable for the development of the disease weakening or death of the seedling may result. It appears that serious injury is largely restricted to the seedling stage. Durrell (*3*, *4*) has found that the diseases caused by Diplodia and Basisporium are not systemic, and the contrary has never been shown for Gibberella.

The primary purpose of the tests herein reported was to establish the general principles concerning the use of seed-corn treatments under farm conditions. The three subjects under investigation are as follows: (1) Effect of field-plot technic on the reliability of seed-treatment results; (2) effect of seed treatments on diseased seed as contrasted with farm-selected "planter-box" seed and disease-free seed; and (3) effect of seed treatment in relation to time of planting, as a protection against soil-borne organisms.

EXPERIMENTAL METHODS

The disinfectants used for the seed-corn treatments were all commercial compounds having some form of mercury as their active disinfectant principle. The materials employed were Uspulun, Bayer Dust, Semesan Jr. or Improved Semesan Jr., and Merko. All of these except Uspulun are dusts to be applied by standard methods at the rate of 2 ounces per bushel of seed. Uspulun is used as a one-fourth of 1 per cent solution in which the seed is soaked for approximately one hour.

The results reported in this paper were all secured at the agricultural experiment station at Lincoln, Nebr., with the exception of some from certain outlying cooperative experiments conducted in 1928. The tests extended over the 4-year period 1925 to 1928, inclusive. Investigations of the first two years, which have already been reported (*11*), were continued and supplemented, and all of the data are here summarized.

In those instances where it was anticipated that stand differences might result from treatment, multiple-row plots were employed to reduce the errors of plot competition, except in the case of certain studies in technic. The plots were commonly 13 rods long and were replicated two or more times. The plantings at the experiment station were done by hand, an equal number of seeds being dropped in each hill, thus permitting accurate field-stand determinations, which were made about three weeks after planting. The seed was spaced about 4 inches apart in the hill, which facilitated the counting of plants and suckers at maturity.

A rather complete set of agronomic notes was taken in connection with these experiments. These data are included in the accompanying tables to indicate the possible effect on growth of the disease or of the seed treatments.

COMPARATIVE EFFECT OF VARIATIONS IN FIELD-PLOT TECHNIC

In order that the results from seed-corn treatment experiments may be applicable, it is necessary that the methods of testing approach closely those that prevail on farms. When the stand of corn in a field is somewhat thinned by seedling diseases, the remaining plants may be expected to produce more heavily because of reduced competition. This increased yield may often largely or entirely compensate for the missing plants. Such adjustment takes place between plants within the same hill or between hills in the same row, or in adjacent rows (*10*). If the character of the field plots used for testing yields is such as to prevent a normal adjustment for missing plants, then correct comparable yields applicable to farm conditions can not be secured.

A special method study was conducted in 1928, comparing treated and untreated Diplodia-infected seed in single rows and in 3-row blocks. In the latter only the middle rows were harvested for yield determinations. A composite lot of diseased seed was divided into four lots. Three portions were treated, with Bayer Dust, Improved Semesan Jr., and Merko, respectively; the fourth remained untreated. The three lots of treated seed were planted consecutively in alternation with untreated seed, the entire series being replicated five times in both kinds of plots. With seed planted in this manner, the test was equivalent to 15 replications of treated and untreated seed in both alternating rows and alternating blocks. The results are given in Table 1.

TABLE 1.—*Comparative results from planting treated and untreated Diplodia-infected seed*[a] *of dent corn in alternating-row and alternating 3-row plots, 1928*

ALTERNATING 1-ROW PLOTS

Treatment	Plots average	Field stand in—		Mature height	Percentage of plants—				Inferior mature plants	Diseased ears[b]	Yield of shelled corn per acre
		Spring	Fall		Barren	Lodged	Broken	With suckers			
	No.	*P. ct.*	*P. ct.*	*In.*					*P. ct.*	*P. ct.*	*Bu.*
Bayer Dust	5	80	79	110	21	2	33	2	4	2	25.5
Improved Semesan Jr.	5	76	75	111	19	1	42	3	1	0	26.3
Merko	5	77	76	110	22	4	43	2	4	1	26.3
Total or average treated	15	78	77	110	21	2	39	2	3	1	26.0
Total or average untreated	15	57	55	109	19	3	40	4	4	2	21.5

ALTERNATING 3-ROW PLOTS[c]

Bayer Dust	5	76	75	108	29	5	51	5	2	2	24.3
Improved Semesan Jr.	5	79	79	109	31	2	37	2	2	1	24.6
Merko	5	75	73	109	26	3	44	1	3	2	24.6
Total or average treated	15	75	76	109	29	3	44	3	2	2	24.5
Total or average untreated	15	59	58	108	13	5	43	4	4	1	25.1

[a] Funk 329 variety obtained from J. R. Holbert of the U. S. Department of Agriculture.
[b] Throughout these tables and discussion "diseased ears" refers to ears visibly affected with dry rot.
[c] Yields based on center row.

In this test it is assumed that correct relative results, free from the systematic error of adjoining plot competition between two unlike

stands, are obtained from the middle rows of 3-row plots. A much better stand resulted from the treated seed, 21 more plants being secured per 100 seeds planted in the single rows and 18 more in the 3-row plots. Expressed in another way: Treatment resulted in 37 and 31 per cent more plants, respectively, in the 1-row plots and the 3-row plots. This did not, however, prove to be of any particular advantage in the multiple-row plots, since the individual plants of the thinner stand were enabled to develop more favorably and thereby still fully occupy the land under the prevailing conditions. The untreated 3-row plots with fewer plants yielded 0.6 bushel, or 2 per cent, more per acre than did the treated plots. On the other hand, a correspondingly thin stand was unable to undergo a corresponding unhampered plant adjustment in the single-row plots because of the close competition with a heavier stand in immediately adjacent rows. Under these highly competitive conditions the heavier stand of the treated plots had an undue advantage, while the thinner stand of the untreated seed was at a marked disadvantage. This resulted in a much exaggerated yield effect in favor of seed treatment. Instead of the 0.6 bushel, or 2 per cent, decrease obtained in the 3-row plots, a 4.5 bushel, or 21 per cent, increased yield was obtained. This difference in favor of treatment represents a systematic experimental error of the test. This competition is also reflected in the effect on the percentage of barren plants under the two methods of testing. Incidentally it may be noted that the further loss in stand during the growing season was practically no greater for the untreated than for the treated seed.

These competition results correspond closely to those obtained by the writer (*10*) in 1914, when two planting rates, two plants and four plants per hill, were likewise compared in alternating rows and alternating 3-row blocks. In the latter case, unhampered by adjoining plot competition with a heavier stand, the 2-plant rate yielded 16 per cent more than the 4-plant rate, whereas in competing single rows the 2-plant rate yielded 18 per cent less. This same principle was exhibited during three years of testing, although the most productive stand naturally varies with the season and variety. The same principle will apply, though in lesser degree, to plots containing two or more rows unless border rows are discarded. Wherever corn is grown at a thick enough planting rate to give maximum yields, this principle of compensation between two adjoining rows differing in stand is quite likely to operate, and for this reason a statement of technic facilitates the interpretation and appraisal of experimental results.

COMPARATIVE EFFECTS OF SEED TREATMENT ON DIPLODIA-INFECTED SEED, FARM-SELECTED SEED, AND NEARLY DISEASE-FREE SEED CORN

TESTS IN 1927

Two lots of Diplodia-infected seed and one lot of nearly disease-free seed were obtained from Illinois for a seed-treatment test in 1927. A portion of each of these lots was treated with Bayer Dust and tested in comparison with untreated seed in duplicate 3-row plots 35 hills long. The corn was planted at the rate of three seeds per hill, which is the standard planting rate in the eastern part of Nebraska. The results are given in Table 2, together with a summary of the results from 17 lots of Nebraska farm-selected seed grown in an adjoining portion of the same field.

TABLE 2.—*Effect of mercuric seed treatment upon the crops from Diplodia-infected, nearly disease-free, and "planter-box" seed corn, 1927*

[Plots consisted of 3 rows 35 hills long, in duplicate]

Source of seed [a]	Description of seed	Treatment	Field stand	Date in tassel	Mature height	Percentage of plants—			Diseased ears	Shrinkage of ear corn	Shelling percentage	Yield of shelled corn per acre
						Barren	Lodged	With suckers				
			Per cent		*Inches*				*Per cent*	*Per cent*		*Bushels*
J. R. Holbert	Diplodia-infected	Bayer Dust	89	July 29	96	3	13	12	1.0	6	83	62.2
		None	80	do	96	4	11	14	.7	4	84	57.9
B. F. Koehler	do	Bayer Dust	85	do	92	5	25	15	1.2	7	84	64.7
		None	79	do	94	3	21	12	1.1	6	84	62.4
J. R. Holbert	Nearly disease-free	Bayer Dust	95	do	93	2	15	36	.8	5	83	66.7
		None	95	do	91	4	10	40	.9	6	83	64.7
17 Nebraska farmers	"Planter-box"	Bayer Dust [b]	85	July 28	87	4	21	20	1.2	6	83	53.9
		None	87	do	87	5	21	21	1.4	6	83	55.0

[a] The laboratory germination tests of this corn in the order listed were 94, 90, 99, and 93 per cent, respectively, for untreated seed.
[b] 8 lots treated with Bayer Dust and 9 with Semesan Jr.; these data for "planter-box" corn taken from Table 4.

One sample of Diplodia-infected seed was improved 9 per cent in stand and 4.3 bushels per acre in yield by treatment, while nearly disease-free seed from the same source was unaffected as to stand but yielded 2 bushels more after treatment. The other sample of Diplodia-infected seed gave a 6 per cent better stand when treated and 2.3 bushels more grain per acre. An average loss in yield of 1.1 bushels per acre resulted from the treatment of farm-selected seed obtained from 17 eastern Nebraska farmers and grown in the same field. The field stand from this seed was improved 2 per cent. While these data are not very conclusive, they indicate that some betterment of stand and yield resulted from the treatment of severely Diplodia-infected seed under the rather favorable conditions of these tests.

TESTS IN 1928

A direct comparision was made in 1928 of the effects of mercuric disinfectants upon Diplodia-infected seed, nearly disease-free seed, and ordinary seed selected by farmers for home use. The tests were systematically replicated four times in 3-row plots of which only the middle rows were harvested for yields. Three seeds were planted in each hill. The results are summarized in Table 3.

Seed treatment increased the field stand 18 per cent for the diseased seed, lowered it 2 per cent for the nearly disease-free seed, and increased it 2 per cent for the farm-selected seed. The loss of plants between spring and fall was almost negligible and differed but little in the lots from treated and untreated seed. Averaging all treatments, the yield of the Diplodia-infected seed was increased 0.7 bushel per acre, as compared with 0.4 bushel decrease for the farm-selected seed and 0.8 bushel decrease for the nearly disease-free seed. There was no evidence of growth stimulation.

Seed treatment had no appreciable effect upon the number of inferior plants or diseased ears produced, even in the case of severe Diplodia-infected seed.

TABLE 3.—*Comparative effects of various mercuric seed treatments upon the crops from Diplodia-infected seed, ordinary "selects bag" and*

TABLE 3.—*Comparative effects of various mercuric seed treatments upon the crops from Diplodia-infected seed, ordinary "planter-box" seed and nearly disease-free seed corn, 1928*

Treatment	Plots averaged	Field stand in—		Date in tassel	Mature height	Percentage of plants—				Inferior plants in—		Diseased ears	Yield of shelled corn per acre [a]
		Spring	Fall			Barren	Lodged	Broken	With suckers	Spring	Fall		
	Number	*Per cent*	*Per cent*		*Inches*					*Per cent*	*Per cent*	*Per cent*	*Bushels*
FUNK 329, DIPLODIA-INFESTED SEED FROM ILLINOIS													
Bayer Dust	4	81	80	Aug. 9	108	28	6	51	7	6	3	2	28.9
Improved Semesan Jr.	4	77	79	do	109	30	2	33	2	7	3	2	27.2
Merko	4	77	76	do	109	24	4	51	1	4	3	2	28.2
Total or average treated	12	78	78	do	109	27	4	45	3	6	3	2	28.1
Total or average untreated	4	60	58	Aug. 10	108	14	5	46	5	7	4	1	27.4
HOGUE YELLOW DENT, "PLANTER-BOX" SEED FROM NEBRASKA													
Bayer Dust	4	92	87	Aug. 5	109	21	8	68	12	5	5	2	31.5
Improved Semesan Jr.	4	93	92	do	107	22	5	63	13	5	3	2	30.2
Merko	4	91	90	do	107	18	7	62	10	4	4	1	32.5
Total or average treated	12	92	90	do	108	20	7	64	12	5	4	2	31.4
Total or average untreated	4	92	92	do	107	23	6	61	7	5	3	1	31.8
FUNK 329, NEARLY DISEASE-FREE SEED FROM ILLINOIS													
Improved Semesan Jr.	4	91	92	Aug. 8	110	26	4	58	5	5	3	1	24.8
Untreated	4	93	92	do	112	24	2	53	7	4	2	1	25.6

[a] Yields were based on the center rows of 3-row plots.

EFFECT OF SEED TREATMENT ON FARM-SELECTED SEED CORN

SEED SECURED FROM 14 COUNTIES IN EASTERN NEBRASKA

In the spring of 1927, 17 samples of farm-selected seed, prepared for home use, were obtained from farmers located in 14 different counties of eastern Nebraska. Each sample was divided into two portions, one being treated with either Bayer Dust or Improved Semesan Jr., as specified, while the other remained untreated. All lots were planted in a field of the experiment station farm which had been in corn the previous year. The plots consisted of three rows 35 hills long. Four consecutive plots were hand-planted to each sample of corn. The first two of these plots were planted, respectively, to treated and untreated seed at the standard rate of three seeds per hill, yields being based on the entire area planted irrespective of the stand secured. The third and fourth plots were likewise planted to treated and untreated seed, but at the rate of six seeds per hill, followed by thinning uniformly to three plants per hill. The thinning of seedlings was done systematically by position in the hill in order to avoid changing the normal proportion of strong and weak seedlings. In this case only those hills were harvested for the yield test which had a full stand.

The corn from all of the farmers was grown in consecutive plots in the field in the same manner. The field stand was determined by counting all seedlings per plot three weeks after planting and calculating their ratio to the number of seeds planted. The average moisture-free weight was determined for those seedlings removed at the time of thinning the one set of plots planted at a double rate.

The results from the two methods of testing are summarized separately in Table 4. It would seem that the general principle relative to need for the treatment of farm-selected seed can be best established by averaging the data from all lots of seed. No great importance can be attached to the results secured with an individual farmer's seed because of a lack of sufficient duplication to permit analysis in such detail. This may be illustrated by the fact that the effect of treatment on the seed of individual farmers ranged from an increase of 3.4 bushels to a decrease of 4.1 bushels in yield per acre. It would seem unjustified to attribute such variation in results to differential varietal response.

As an average for the 17 samples of farm-selected corn, planted at the rate of three seeds per hill, the untreated seed yielded 55 bushels and the treated seed 53.9 bushels per acre. Where the seed had been planted at a double rate and thinned to a normal stand of three plants per hill, the untreated seed averaged 56.9 bushels and the treated 56.5 bushels per acre. At the age of 3 weeks, the field stand averaged three more plants per 100 seeds planted for the untreated than for the treated seed. No material effects from treatment were observed on seedling vigor, date in tassel, mature stalk height, barren stalks, lodged stalks, suckers, 2-ear stalks, unsound ears, shrinkage of ear corn, or shelling percentage.

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TABLE 4.—*Summary of the effect of mercuric seed treatments upon the growth and yield of farm-selected "planter-box" seed corn obtained from 17 farmers located in 14 counties of eastern Nebraska, 1927*

PLANTED THREE SEEDS PER HILL

[Plots consisted of three rows 35 hills long, in duplicate]

Treatment	Seed sources averaged	Field stand	Seedlings, 3 weeks old		Date in tassel	Mature height	Percentage of plants—				Diseased ears	Shrinkage of ear corn	Shelling percentage	Yield of shelled corn per acre
			Average weight	Average height			Barren	Lodged	With suckers	With 2-eared stalks				
	Number	*Per cent*	*Grams*	*Inches*		*Inches*					*Per cent*	*Per cent*		*Bushel*
Improved Semesan Jr.	9	84			July 29	89	4	23	23	3	1.7	7	82	54.2
Untreated	9	87			do	88	4	25	23	2	.8	6	83	55.5
Bayer Dust	8	86			July 27	85	4	18	18	2	.8	5	83	53.5
Untreated	8	88			July 28	86	5	16	19	2	2.0	7	83	54.5
Total or average treated	17	85			do	87	4	21	20	2	1.2	6	83	53.9
Total or average untreated	17	87			do	87	5	21	21	2	1.4	6	83	55.0
	PLANTED SIX SEEDS PER HILL AND THINNED TO THREE PLANTS PER HILL													
Improved Semesan Jr.	9	82	0.86	13.3	July 29	87	4	24	16	2	1.3	6	83	59.5
Untreated	9	85	.82	13.2	July 28	87	3	24	20	2	1.2	6	83	59.1
Bayer Dust	8	84	.94	13.8	do	86	4	27	17	1	1.3	6	83	53.5
Untreated	8	86	.90	13.6	do	84	3	26	14	2	2.0	4	83	54.6
Total or average treated	17	83	.90	13.6	July 29	87	4	26	17	2	1.3	6	83	56.5
Total or average untreated	17	86	.86	13.4	July 28	86	3	25	17	2	1.6	5	83	56.9

SEED SECURED FROM 20 LANCASTER COUNTY FARMERS AND TESTED INDIVIDUALLY

Seed corn collected from 20 Lancaster County, Nebr., farmers was included in seed-treatment tests at the experiment station in 1926 and 1927. Uspulun-treated and untreated seed of each man's corn were grown side by side in unduplicated 1-row plots 64 hills in length at the rate of 3 plants per hill. Moisture conditions were much too dry for corn in 1926 but very favorable in 1927.

TABLE 5.—*Average effect of seed treatment with Uspulun upon the growth and yield of dent seed corn secured from 20 Lancaster County, Nebr., farmers and tested individually, 1926 and 1927*

[Single-row plots, 64 hills in length]

Plant character	Results in 1926 with—		Results in 1927 with—		Average	
	Untreated seed	Treated seed	Untreated seed	Treated seed	Untreated seed	Treated seed
Plots.....number	20	20	20	20	20	20
Field stand.....per cent	75	77	76	79	76	78
Plant height June 7.....inches	8.6	8.3				
Mature height.....do	74	74	95	95	85	85
Barren plants.....per cent	71	77	5	5	38	41
Lodged plants.....do	17	15	13	15	15	15
Suckers.....do	6	4	6	5	6	5
Shrinkage of ear corn.....do	11	12	10	9	11	11
Shelling percentage	80	80	84	84	82	82
Diseased ears.....per cent			2	2		
Yield per acre.....bushels	7.7	7.6	70.2	70.7	39	39.2

The average results (Table 5) for the seed secured from all of the farmers are again regarded as the most reliable indication of the effects of seed treatment. Such averages for the two years show yields per acre of 39.2 bushels for the treated and 39 bushels for the untreated seed. A 2 per cent better field stand from treated seed in this test is offset by a 3 per cent decrease in the preceding test.

No significant differences are shown in the averages for barren plants, suckers, lodged plants, plant height, shrinkage of ear corn, shelling percentage, or percentage of unsound ears in the crop.

SEED SECURED FROM 30 LANCASTER COUNTY FARMERS AND PLANTED AS A MIXTURE

During each of the three years 1925–1927 seed selected for home use was secured from 30 Lancaster County, Nebr., farmers and mixed together for a general comparative test of the three commercial organic mercury compounds then available. Six systematically distributed 3-row plots, 64 hills long, were planted to each treatment during each season. The annual and average results are given in Table 6. Although the growth and yield varied greatly in the different years, no material effect from seed treatment was observed in any season. The differences for the various compounds were small and well within the limits of experimental error.

TABLE 6.—*Effect of various seed treatments upon the growth and yield of "planter-box" seed corn secured from 30 different Lancaster County, Nebr., farmers and planted in composite, 1925–1927*

[Plots consisted of 3 rows 64 hills long]

RESULTS FOR 1925

Treatment	Plots averaged	Field stand secured	Seedling height June 7	Mature height	Percentage of plants—			Ear corn		Yield of shelled corn per acre
					Barren	Lodged	With suckers	Shrinkage	Shelling	
	Number	*Per cent*	*Inches*	*Inches*				*Per cent*	*Per cent*	*Bushels*
Uspulun	6	79		98	15	25	6	6	83	39.2
Semesan Jr.	6	79		99	16	20	5	9	83	39.5
Bayer Dust	6	79		98	15	21	7	8	82	38.7
Total or average treated	18	79		98	15	22	6	8	83	39.1
Total or average untreated	6	79		98	15	22	7	7	83	39.4

RESULTS FOR 1926

Uspulun	6	74	8.4	74	68	18	5	11	79	7.4
Semesan Jr.	6	74	8.6	74	68	19	5	11	80	6.9
Bayer Dust	6	72	8.5	73	60	19	7	11	80	7.3
Total or average treated	18	73	8.5	74	65	19	6	11	80	7.2
Total or average untreated	6	73	8.5	73	63	20	5	8	80	7.2

RESULTS FOR 1927

Uspulun	6	78		99	5	15	6	11	84	71.5
Semesan Jr.	6	74		100	4	13	6	13	84	70.6
Bayer Dust	6	75		101	4	16	7	13	85	72.7
Total or average treated	18	76		100	4	15	6	12	84	71.6
Total or average untreated	6	77		99	3	13	5	14	85	70.7

AVERAGE FOR THREE YEARS

Uspulun	18	77		90	29	19	6	9	82	39.4
Semesan Jr.	18	76		91	29	17	5	11	82	39.0
Bayer Dust	18	75		91	26	19	7	11	82	39.6
Total or average treated	54	76		91	28	18	6	10	82	39.3
Total or average untreated	18	76		90	27	18	6	10	83	39.1

On the basis of a 3-year average, the field stands secured from seed treated with Uspulun, Bayer Dust, Semesan Jr., and from untreated seed were 77, 75, 76, and 76 per cent, respectively, while the corresponding yields of shelled corn were 39.4, 39.6, 39.0 and 39.1 bushels per acre. Averaging all treatments, the yield was reduced 0.3 bushel in 1925, was unaffected in 1926, and was increased 0.9 bushel in 1927. This amounts to 0.2 bushel increase for all treatments during the three years. The average stand for the three years was the same from treated and untreated seeds.

EFFECT OF SEED TREATMENT ON THE YIELD OF FARM-SELECTED CORN WHEN TESTED IN DIFFERENT LOCALITIES

Seed corn obtained from seven different eastern Nebraska farmers was compared for yield with and without treatment on the experiment station farm at Lincoln and also 60 miles distant, at Valley, Nebr. The point of special interest in this test is the comparatively more favorable environment at Valley in respect to rainfall and subirrigation. At the experiment station the corn was planted by hand at the rate of three seeds per hill in duplicate 3-row plots. At Valley the corn was planted in duplicate 2-row plots by means of a corn planter set at a constant calibration. In all cases the treated and untreated seeds of each farmer was planted in adjacent plots.

TABLE 7.—*Comparative results from the planting of treated "planter-box" dent corn seed at Lincoln and at Valley, Nebr., 1928* [a]

Treatment	Field stand	Plants per 20 rods	Percentage of plants—			Diseased ears	Yield of shelled corn per acre
			Smutty	Barren	Lodged		
RESULTS AT LINCOLN, NEBR.							
	Per cent	*Number*				*Per cent*	*Bushels*
Bayer Dust	95	269	17	32	62	3	27.5
Untreated	96	272	15	33	59	2	27.3
RESULTS AT VALLEY, NEBR.							
Bayer Dust		219				0.6	62.3
Untreated		223				.2	62.7

[a] This test was conducted in cooperation with the Agricultural Extension Service. The seed was collected from farmers in four different eastern Nebraska counties. Results represent averages for seed from seven farmers.

The average results are reported for each locality in Table 7. Practically identical stands and yields were secured at Lincoln from both treated and untreated seed. At Valley the application of dust to the seed reduced the rate of drop, and on an average approximately 2 per cent fewer plants grew in the treated than in the untreated plots. Treatment lowered the yield 0.4 bushel per acre.

For use in testing the effects of three different dust treatments at Valley, Nebr., in 1928, the seed from these seven farmers was mixed in equal proportions. This seed mixture was divided into four lots, three of which were treated, respectively, with Bayer Dust, Improved Semesan Jr., and Merko; the fourth remained untreated. The untreated seed was planted in alternation with the three lots of treated seed in 2-row plots by means of a corn planter. The three treated lots averaged 5 per cent lower stand and 3.3 bushels lower yield than the untreated seed. (Table 8.) The reduced yield may perhaps have been due in part to the effect of plant competition associated with the manner of testing here followed. Since the treatment affected the mechanical operation of the planter, thereby lowering the stand 5 per cent, error due to plot competition might be expected, as was pointed out in connection with Table 1.

TABLE 8.—*Effect of various seed-corn treatments upon the yield of ordinary farm-selected "planter-box" dent corn seed[a] planted at Valley, Nebr.,[b] 1928*

[The plots consisted of 2 rows, 10 rods long, and were planted in duplicate with a corn planter set at a constant drop]

Treatment	Plants per 20 rods	Diseased ears	Shelling percentage	Shrinkage of ear corn	Yield of shelled corn per acre
	Number	*Per cent*		*Per cent*	*Bushels*
Bayer Dust	212	0	82.9	6.7	56.4
Semesan jr.	215	0	81.3	8.1	55.4
Merko	191	0	80.7	7.3	54.9
Average	206	0	81.6	7.4	55.6
Untreated	216	0	81.3	7.7	58.9

[a] This corn was a composite sample of "planter-box" seed from 7 farmers.
[b] This test was conducted in cooperation with the Agricultural Extension Service on the farm of J. L. Gilmore.

PROTECTION OF SEED AGAINST SOIL-BORNE ORGANISMS

It has recently been suggested by a number of workers that the use of these mercuric dusts on seed corn will protect the seed against soil-borne organisms which may cause rotting in the early spring, and universal treatment for this purpose is recommended. Very few data bearing on this question have, however, been published.

In order to secure information concerning this problem a combined date-of-planting and seed-treatment test was made during the 3-year period 1926 to 1928, inclusive. Farm-selected seed of two standard eastern Nebraska varieties, White Prize and Hogue Yellow Dent, were each treated with Uspulun during 1926 and 1927, and with Bayer Dust in 1928. Treated and untreated seed of each variety was planted in adjacent 2-row plots at rather uniform 10-day intervals, beginning either on the 15th or 25th of April, and ending June 15. The entire series was in duplicate. Since the normal planting season for this region extends from about May 5 to May 20, it is evident that the dates here included materially preceded and followed the usual time of planting. The object of this test was to determine whether the results of treatment might be influenced by the date of planting with the usual accompanying change in climatic conditions.

TABLE 9.—*Comparative field stand secured from treated and untreated "planter-box" seed corn of two standard varieties, planted at seven different dates in the spring, 1926–1928*

[Plots consisted of 2 rows 64 hills long, in duplicate]

RESULTS FOR 1926

Variety	Treatment	Percentage field stand secured from seed planted on the average planting dates indicated							Average stand [a]	Laboratory germination test
		Apr. 15	Apr. 25	May 5	May 15	May 25	June 5	June 15		
									Per cent	*Per cent*
White Prize	Uspulun	87	86	87	83	76	88	72	83	98
	Untreated	87	87	86	83	77	85	72	82	97
Hogue	Uspulun	79	82	81	81	80	75	69	78	93
	Untreated	80	80	83	82	80	74	68	78	94
Average	Uspulun	83	84	84	82	78	82	71	81	96
	Untreated	84	84	85	83	79	80	70	80	96

RESULTS FOR 1927

White Prize	Uspulun	67	63	64	77	69	61	67	67	88
	Untreated	64	58	67	75	66	59	65	65	91
Hogue	Uspulun	69	69	65	77	67	65	69	69	93
	Untreated	68	69	71	77	66	66	70	70	95
Average	Uspulun	68	66	65	77	68	63	68	68	91
	Untreated	66	64	69	76	66	63	68	68	93

RESULTS FOR 1928

White Prize	Bayer Dust	84	88	75	83	82	84	83	83	99
	Untreated	79	85	79	83	82	81	80	81	98
Hogue	Bayer Dust	89	92	80	92	89	89	88	88	98
	Untreated	87	90	83	90	87	87	84	87	100
Average	Bayer Dust	87	90	78	88	86	87	86	86	99
	Untreated	83	88	81	87	85	84	82	84	99

AVERAGE FOR 3 YEARS

White Prize	Treated	80	75	77	78	80	72	77	77	95
	Untreated	79	74	78	78	77	70	76	76	95
Hogue	Treated	81	77	79	82	77	74	78	78	95
	Untreated	79	78	81	81	76	73	78	78	96
Average	Treated	81	76	78	80	79	73	78	78	95
	Untreated	79	77	80	80	77	72	77	77	96

[a] Averages for last 6 planting dates only.

TABLE 10.—*Comparative yields secured from treated and untreated "planter-box" seed corn of two standard varieties, planted at seven different dates in the spring, 1926–1928*

[Plots consisted of 2 rows 64 hills long, in duplicate.]

RESULTS FOR 1926

Variety	Treatment	Yields in bushels of shelled corn per acre secured from seed planted on the average planting dates indicated							Average yield *
		Apr. 15	Apr. 25	May 5	May 15	May 25	June 5	June 15	
									Bushels
White Prize	Uspulun	5.3	6.0	6.2	9.9	14.5	19.4	13.4	11.6
	Untreated	6.0	5.1	6.8	10.5	14.4	21.5	13.5	12.0
Hogue	Uspulun	7.3	7.8	8.6	9.6	13.1	22.6	19.7	13.6
	Untreated	6.6	5.9	7.1	11.6	14.0	25.1	19.3	13.8
Average	Uspulun	6.3	6.9	7.4	9.8	13.8	21.0	16.5	12.6
	Untreated	6.3	5.5	7.0	11.1	14.2	23.3	16.4	12.9

RESULTS FOR 1927

White Prize	Uspulun	45.1	48.4	43.5	42.6	40.2	36.4	42.7
	Untreated	45.8	46.4	45.7	42.8	38.0	36.1	42.5
Hogue	Uspulun	50.2	49.2	48.9	51.8	49.8	44.8	49.1
	Untreated	50.5	48.6	49.3	51.2	45.9	43.4	48.2
Average	Uspulun	47.7	48.8	46.2	47.2	45.0	40.6	45.9
	Untreated	48.2	47.5	47.5	47.0	42.0	39.8	45.3

RESULTS FOR 1928

White Prize	Bayer Dust	50.6	55.7	45.0	41.9	37.0	31.6	36.7	40.3
	Untreated	51.9	57.4	50.3	45.5	40.2	30.7	30.4	42.4
Hogue	Bayer Dust	58.1	59.4	64.7	55.0	45.9	41.6	37.2	50.6
	Untreated	56.0	65.0	64.4	50.6	41.1	44.6	36.3	50.3
Average	Bayer Dust	54.4	57.5	54.9	48.5	41.5	36.6	34.0	45.5
	Untreated	54.0	61.2	57.4	48.1	40.7	37.7	33.4	46.5

AVERAGE FOR 3 YEARS

White Prize	Treated	35.6	33.2	31.8	31.4	30.4	26.8	31.5
	Untreated	36.1	34.5	33.9	32.5	30.1	26.7	32.3
Hogue	Treated	39.1	40.8	37.8	36.9	38.0	33.9	37.8
	Untreated	40.5	40.0	37.2	35.4	38.5	33.0	37.5
Average	Treated	37.4	37.0	34.8	34.2	34.2	30.4	34.7
	Untreated	38.3	37.3	35.6	34.0	34.3	29.9	34.9

* Averages for the last 6 planting dates only.

TABLE 11.—*Effect of seed treatments upon the performance of "planter-box" seed corn planted at six dates in the spring during three years*

[Plots consisted of two rows 64 hills long, in duplicate. Averages for Hogue and White Prize varieties, 1926–1928]

Treatment and date of planting	Field stand	Date in tassel	Plant height	Percentage of plants—				Unsound ears	Shrinkage of ear corn	Shelling percentage	Yield of shelled corn per acre
				Smutted	Barren	2-eared	Broken or lodged				
Untreated:	*Per cent*		*Inches*					*Per cent*	*Per cent*		*Bushels*
Apr. 25, 1926	84	July 21	73	29	50	2	77	.....	8	70	5.5
Apr. 25, 1927	63	July 27	90	9	3	3	11	2	8	83	48.2
Apr. 25, 1928	88	July 22	107	5	3	3	46	1	5	85	61.2
Average	78	July 23	90	14	19	3	45		7	79	38.3
Treated:											
Apr. 25, 1926	84	July 21	70	31	32	4	72	.....	12	80	6.9
Apr. 25, 1927	68	July 28	87	7	1	3	11	1	6	85	47.7
Apr. 25, 1928	90	July 22	107	6	4	2	43	1	3	85	57.5
Average	81	July 24	88	15	12	3	42	.....	7	83	37.4
Untreated:											
May 5, 1926	85	July 29	67	28	40	6	74	.....	10	78	7.0
May 5, 1927	64	Aug. 2	90	8	2	4	10	2	6	83	47.5
May 5, 1928	81	July 26	104	6	2	3	43	1	4	84	57.9
Average	77	July 29	87	14	15	4	42	.....	7	82	37.5
Treated:											
May 5, 1926	84	July 29	68	32	45	4	74	.....	10	78	7.4
May 5, 1927	66	Aug. 2	90	6	3	3	10	0	8	86	48.8
May 5, 1928	78	July 26	104	5	2	3	46	1	4	84	54.9
Average	76	July 29	87	14	17	3	43	.....	7	83	37.0
Untreated:											
May 14, 1926	83	Aug. 1	69	26	27	5	69	.....	10	75	11.1
May 14, 1927	69	Aug. 4	91	8	2	2	11	2	7	84	47.5
May 14, 1928	87	Aug. 1	111	3	3	2	43	0	3	86	48.1
Average	80	Aug. 2	90	12	11	3	41	.....	7	82	35.6
Treated:											
May 14, 1926	82	Aug. 1	66	24	28	5	78	.....	10	78	9.8
May 14, 1927	65	Aug. 4	90	6	3	2	11	3	7	83	46.1
May 14, 1928	88	Aug. 1	111	6	3	1	42	0	7	85	48.5
Average	78	Aug. 2	89	12	11	3	44	.....	8	82	34.8
Untreated:											
May 25, 1926	79	Aug. 6	73	25	20	4	72	.....	12	81	14.2
May 25, 1927	76	Aug. 10	91	7	2	6	6	2	9	83	47.0
May 25, 1928	85	Aug. 6	114	6	4	1	51	2	8	84	40.7
Average	80	Aug. 7	93	13	9	4	43	.....	10	83	34.0
Treated:											
May 25, 1926	78	Aug. 6	70	23	17	5	66	.....	11	82	13.8
May 25, 1927	77	Aug. 15	92	7	1	5	4	1	11	82	47.2
May 25, 1928	86	Aug. 6	114	5	6	1	52	1	7	83	41.5
Average	80	Aug. 9	92	12	8	4	41	.....	10	82	34.2
Untreated:											
June 5, 1926	80	Aug. 12	71	20	8	4	67	.....	13	83	23.3
June 5, 1927	66	Aug. 14	92	4	2	4	6	3	14	81	42.0
June 5, 1928	84	Aug. 12	101	4	5	2	32	2	11	83	37.7
Average	77	Aug. 13	88	9	5	3	35	.....	13	82	34.3
Treated:											
June 5, 1926	82	Aug. 12	73	18	7	4	66	.....	11	81	21.0
June 5, 1927	68	Aug. 14	89	3	1	5	4	3	12	82	45.0
June 5, 1928	87	Aug. 12	101	5	5	2	37	2	13	84	36.6
Average	79	Aug. 13	88	9	4	4	36	.....	12	82	34.2

TABLE 11.—*Effect of seed treatments upon the performance of "planter-box" seed corn planted at six dates in the spring during three years*—Continued

Treatment and date of planting	Field stand	Date in tassel	Plant height	Percentage of plants—				Unsound ears	Shrinkage of ear corn	Shelling percentage	Yield of shelled corn per acre
				Smutted	Barren	2-eared	Broken or lodged				
Untreated:	*Per cent*		*Inches*					*Per cent*	*Per cent*		*Bushels*
June 15, 1926	70	Aug. 16	76	13	10	4	40	.....	17	79	16.4
June 15, 1927	63	Aug. 25	101	4	1	2	6	2	19	80	39.8
June 15, 1928	82	Aug. 18	101	6	9	2	32	2	15	82	33.4
Average	72	Aug. 20	93	8	7	3	26	.....	17	80	29.9
Treated:											
June 15, 1926	71	Aug. 16	76	13	6	4	40	.....	16	76	16.5
June 15, 1927	63	Aug. 25	96	4	1	4	7	2	20	81	42.0
June 15, 1928	86	Aug. 18	101	4	5	2	32	1	20	83	34.0
Average	73	Aug. 20	91	7	4	3	26	.....	19	80	30.8
Untreated:											
1926 Average	80	Aug. 4	72	24	26	4	67	.....	12	78	12.9
1927 Average	65	Aug. 9	93	7	2	4	8	2	11	82	45.3
1928 Average	86	Aug. 4	106	5	4	2	41	1	8	84	46.5
Average	77	Aug. 6	90	12	11	3	39	.....	10	81	34.9
Treated:											
1926 Average	80	Aug. 4	71	24	23	4	66	.....	12	79	12.6
1927 Average	68	Aug. 10	91	6	2	4	8	2	11	83	46.1
1928 Average	86	Aug. 4	106	5	4	2	42	1	9	84	45.5
Average	78	Aug. 6	89	12	10	3	39	.....	11	82	34.7

TABLE 12.—*Maximum, minimum, and mean daily air temperatures in °F.[a] during 10-day periods following the corn-planting dates reported in Tables 9, 10, and 11; 1926, 1927, and 1928*

Item	Average daily air temperature on dates included in period						
	Apr. 15 to Apr. 24	Apr. 25 to May 4	May 5 to May 14	May 15 to May 24	May 25 to June 4[b]	June 5 to June 14	June 15 to June 24
1926:							
Maximum	69.8	76.1	67.6	79.8	83.2	87.2	77.3
Minimum	42.7	46.8	51.6	54.0	60.0	62.8	56.4
Mean	56.2	61.4	59.9	66.6	71.5	75.0	67.0
1927:							
Maximum	56.4	73.6	67.7	78.0	71.2	75.5	79.2
Minimum	40.0	50.5	46.5	56.4	51.8	55.9	59.3
Mean	48.1	62.0	57.2	67.1	61.5	65.7	69.2
1928:							
Maximum	58.3	70.1	76.2	76.4	75.6	76.5	78.4
Minimum	34.3	42.4	51.5	54.3	53.5	55.6	55.6
Mean	46.3	56.1	63.9	65.3	64.6	66.0	66.4
Normal mean	53.3	58.2	59.5	62.8	66.3	70.9	72.8

[a] These temperature data are of interest in connection with date-of-planting tests reported in Tables 9, 10, and 11.
[b] 11-day period.

The field stands secured at each planting date are reported separately for each variety and are also summarized for the 3-year period in Table 9. The corresponding acre yields of shelled corn are reported

in Table 10. The results secured each year with respect to various agronomic characters of the crop are summarized for the different planting dates in Table 11. The maximum, minimum, and mean daily air temperatures that prevailed during the 10-day periods immediately following the dates of planting in 1926 to 1928 are shown in Table 12.

The maximum deviation in stand obtained on any one planting date ranged from an increase of 5 per cent to a decrease of 6 per cent following treatment. When both varieties were averaged for the three years, the maximum deviation in stand between treated and untreated seed on any one planting date was 2 per cent, with a grand average of 1 per cent in favor of treatment. Considering the 6 dates as an average for the three years, the treatment gave a better stand on 3 dates, a reduced stand on 2 dates, and had no effect on 1 date.

Considering the grain yields for the individual years at the various dates, the maximum deviation ranged from an increase of 4.8 bushels per acre in favor of treatment to a decrease of 5.6 bushels per acre, likewise following treatment. Averaging both varieties for the three years, the maximum deviation in yield between treated and untreated seed at any one planting date was 1.2 bushels, with a grand average of 0.2 bushel in favor of the untreated seed. By comparing the individual dates averaged for the three years, the treated corn was found to yield most for the fifth and seventh and least for the other planting dates.

It is apparent from Table 11 that almost identical results were obtained from treated and untreated seed with respect to such characters as date of tasseling, plant height, barrenness, lodging, unsound ears, shrinkage and shelling percentage of ear corn after husking, percentage of field stand, and yield of grain per acre. From these data it does not appear that these mercuric compounds serve in an important degree as a protection of the seed from soil-borne organisms.

Smut counts, included in Table 11, give further evidence that corn smut is not influenced by seed treatment. Similar results from smut counts were obtained in all of the experiments herein reported.

DISCUSSION

The question may arise whether the conditions that influence the development of seedling diseases at the experiment station are representative of those that generally prevail in this State. Results with viable seed corn known to be heavily infected with specific ear-rot organisms indicate injurious effects on stand corresponding with those reported for Illinois and Iowa, where both climate and soil are regarded as conducive to the development of these organisms. A comparative field-plot test with such seed systematically replicated 30 times, in 1928, showed an average increase of 18 per cent in field stand as a result of treatment with mercuric fungicides. (Table 1.) In another test in 1928 (Table 2) the stand from nearly disease-free seed had 52 per cent more plants than the stand from Diplodia-infected seed of the same variety. In 1927 duplicate tests gave average field stands of 95 and 80 per cent, respectively, for nearly disease-free and Diplodia-infected seed. Treatment of the diseased seed improved the stand 9 per cent.

In 1922 a comparative replicated field test made by the writer (*10*) showed typical inferior stands when diseased seed was used, as follows: Diplodia-infected seed and Fusarium-infected seed gave field stands only 64 and 96 per cent as large as those from nearly disease-free seed. These results again were typical of those that have commonly been described for the State of Illinois, and lend confidence to the belief that these Nebraska Experiment Station conditions are responsive to both the disease organisms and to the mercuric fungicides. It is concluded that results obtained from seed treatments at the experiment station are applicable elsewhere in the State.

It is evident that inferior stands may be expected from seed that is severely infected with dry-rot seedling-disease organisms. It is also apparent that the loss of stand resulting from the use of such seed may be materially lessened through treatment with some one of the mercuric seed-corn disinfectants. However, such infected seed, even though treated, is likely to be definitely inferior, from the standpoint of field stand, to untreated nearly disease-free seed and to representative farm-selected seed. Whether the differences in stand due to either the disease or the treatment have any material effect upon yield depends upon the season and the actual stand obtained. This phase of the problem has been constructively analyzed by Clayton (*1*) in connection with a review of experiments pertaining to Diplodia dry rot of corn. He concludes that no differences in yield resulted from the use of diseased seed when it was planted thick enough to compensate for the reduced germination.

In view of the inferior stands secured in 1927 and 1928 from seed infected with Diplodia and in 1922 from seed infected with Diplodia and Fusarium (*10*), it becomes apparent that the germinator test described by the writer (*9, 11*) and patterned after that of Holbert and Hoffer (*7*) was unreliable as an index of infection with dry-rot disease organisms. In these earlier germinator tests a large number of seed ears were classified as diseased and disease-free without an attempt being made to identify the disease organisms. When planted, both groups gave approximately equal stands and equal yields during a 6-year period.

The failure to secure improved stands or improved yields during the last four years from the treatment of representative farm-selected seed collected from a large number of Nebraska farmers is evidence that these seedling dry rots are exerting little influence as seed-borne diseases in this State. This is quite at variance with the conclusions reached in Iowa by Melhus, Reddy, Raleigh, and Burnett (*12*), who show increases of 2.2 to 4.6 bushels per acre from the use of these commercial dust treatments applied to a local sample of farm-selected seed in each of five counties of the State in 1927. In Illinois also increases ranging from 2.1 to 5.4 bushels are reported for well-selected but untested seed, by Holbert, Reddy, and Koehler (*8*).

The failure of field stands and yields secured from farm-selected seed to be materially benefited by mercuric seed treatments irrespective of the time or favorableness of planting conditions is again at variance with the conclusions reached in Iowa and Illinois by the investigators last cited.

If the various results for these three States are typical, it follows that farm-selected seed planted in Nebraska is less subject to infec-

tion with dry-rot seedling-disease organisms than is farm-selected seed in Iowa and Illinois. Climatic and soil conditions in Nebraska are perhaps also less conducive to development of the seed-borne and soil-borne organisms which attack the planted seed and the young seedling. It is not known to what extent differences in plat technic may have influenced the relative yields where stands were affected by seed selection or treatment. That results and conclusions may vary with the experimental procedure has here been demonstrated.

It may be concluded that Nebraska-grown seed ears selected on the basis of sound and mold-free appearance are generally not appreciably benefited in production by treatment with these mercuric fungicides.

SUMMARY

Four commercial mercuric seed-corn treatments were tested under field conditions at the Nebraska Agricultural Experiment Station during the 4-year period 1925 to 1928, inclusive. These consisted of one liquid fungicide, Uspulun, and three dust compounds, Bayer Dust, Semesan Jr., or Improved Semesan Jr., and Merko. Field conditions at the station proved favorable to the development of the Diplodia seedling disease of corn when severely infected seed was planted, but at least partial control followed the treatment of such seed, the various treatments giving similar results. On the other hand farm-selected seed obtained from Nebraska growers was not significantly benefited by treatment.

Conclusions reached by a number of investigators elsewhere regarding the nonsystemic nature of the Diplodia dry-rot disease were substantiated in 1928 by direct comparison of the seed value of Diplodia-infected seed and nearly disease-free seed selected from the same variety. Although the seed infection curtailed the field stand 35 per cent, it caused no significant deterioration in plants or serious losses between spring and maturity. It did not greatly affect the growth of the plants or the proportion of inferior plants or diseased ears produced. The acre yield from the diseased seed was 1.8 bushels greater, probably due to the advantage of a somewhat thinner stand.

In a thoroughly replicated test in 1928, the three dust compounds improved the field stand from Diplodia-infected seed on an average 18 per cent and lowered the yield per acre half a bushel when tested in multiple-row plots. Failure to increase yield or to materially influence the growth, health, or grain quality of the surviving plants is evidence that the benefit from these seed treatments was limited to the germination and seedling stage of development. In a corresponding test made in alternating single-row plots, the same general results were obtained except that the treated seed yielded 4.5 bushels per acre more than the untreated. This difference in effect on yield as related to character of test plot, represents an experimental error of 5 bushels due to plot competition between unlike stands in the case of the 1-row plots. This suggests the necessity for knowing the details of plot technic in order that data may be properly appraised.

In 1927 seed treatment increased the field stands of two lots of Diplodia-infected seed an average of 8 per cent and the grain yield an average of 3.3 bushels per acre. No other significant effects were noted.

A total of 141 samples of farm-selected seed were tested for response to seed treatment during the 4-year period. Planting was done each

year during the normal planting season except in a special test during three years in which the seven planting dates materially overlaped the normal season. When the effects of treatment as summarized for the various experiments with farm-selected seed are prorated in accordance with the number of samples of corn represented, it is found that the field stand was increased an average of 0.28 per cent and the yield was increased 0.03 bushel per acre. This slight response to the mercuric seed disinfectants shows how little damage results from dry-rot infection of farm-selected seed under normal conditions in Nebraska. There have been no significant practical effects from the seed treatment of 141 samples of farm-selected corn upon such characters as stand, vigor and size of growth, earliness of maturity, percentages of smutty, barren, lodged, broken, and diseased plants, percentage of diseased ears, shrinkage and shelling percentage of the ear corn, and yield of grain per acre.

No beneficial effect was found to result from disinfecting seed corn to protect it from soil-borne organisms under field conditions associated with both abnormally early and late planting.

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UTILIZATION OF CALCIUM BY THE GROWING CHICK[1]

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INTRODUCTION

Considered from the quantitative standpoint, calcium is the most important mineral element involved in chick nutrition. With relatively little effort the most common forms of calcium can be changed to other forms should these be found to serve the purposes of nutrition more efficiently. The fundamental problem with reference to calcium is therefore one of obtaining proper assimilation and fixation. Calcium salts, whether soluble or insoluble, appear to be fixed in the bone tissue with considerable difficulty and our problem is one not simply of providing calcium but rather of providing it in a form and in combination with other factors which permit absorption and deposition. This paper presents the results of a study of the assimilation and utilization of calcium by growing chicks when this element was furnished in several different forms.

EXPERIMENTAL PROCEDURE

As a basal ration for the chicks used in the experiments reported in this paper a mixture was chosen that included corn, wheat, and milk proteins. This ration has many points in common with the mixtures used by thousands of practical poultry producers. Yeast and cod-liver oil were added to insure a sufficient supply of vitamins B and D, and sunshine was available to all lots in a screen-inclosed concrete-floored runway in front of each brooding unit. Steam and electric brooders were used for maintaining the proper temperatures for growth. The basal ration employed in the first series of experiments consisted of the following: Yellow corn meal, 50 per cent; shorts, 24 per cent; casein, 10 per cent; blood meal, 5 per cent; yeast, 3 or 4 per cent as indicated for each series; cod-liver oil, 2 per cent; and starch, 5 or 6 per cent as indicated for each series.

The starch was included in the above ration so that additions of minerals could be made by replacing an equivalent amount of starch without changing the vitamin or protein plane. This basal ration is very low in ash elements, containing only 0.174 per cent of calcium and 0.495 per cent of phosphorus. It was planned to be complete with respect to proteins, vitamins A, B, and D, and was very palatable, so that all requirements for energy were easily satisfied. The protein plane of the basal ration was 23.75 per cent. It was offered as an all-mash ration, available to the birds at all times. Ordinary tap water was used, and this contributed a small amount of calcium in addition to that contained in the ration. The water contained from 50 to 60 parts of CaO per million, and from observations made

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it is estimated that about 3,600 gm. of water was consumed per chick during the 8-week period of each experiment. About 0.18 gm. of calcium per chick may therefore have been added to the available supply from this source.

Single-comb White Leghorn chicks hatched from the college flocks were used for these experiments. Each chick was banded and weighed individually at the beginning of the experiment and biweekly thereafter. All other environmental conditions except length of day were standardized, but since growth rate is very markedly influenced by the length of day, no attempt is made to compare the growth rates of any chicks other than those which were fed at the same time in parallel pens in the chick nutrition laboratory.

Sex was determined on all lots sufficiently developed sexually to make accurate determinations possible. The growth curves in the graphs represent weighted averages of both sexes. This seemed to be

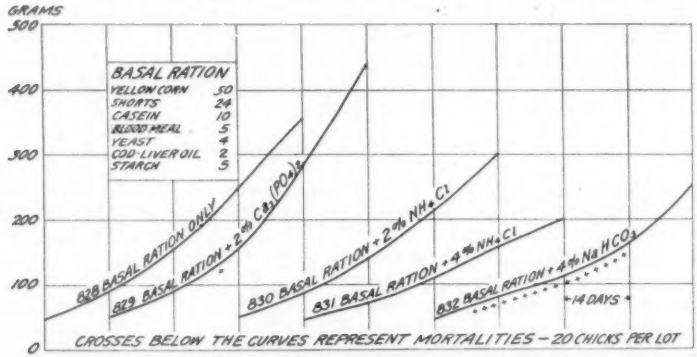

FIGURE 1.—Growth of chicks increased by a calcium supplement and inhibited by the addition of certain other minerals to the basal ration

the most accurate method of presenting the results. From 20 to 40 chicks were used in each lot, the same number of chicks being used in each pen in each series of experiments.

MINIMUM CALCIUM REQUIREMENTS

Until quite recently the prevailing opinion with reference to the mineral requirements of the growing chick has been that safety lies in providing an excess of essential mineral constituents. The soundness of this conclusion has however, been disproved by the experiments of Mussehl, Blish, and Ackerson (*5*).[2] Anticipating that evidence would be obtained of disturbed metabolism because of mineral deficiencies in the basal ration, even when vitamin D and radiant energy requirements were satisfied, the writers placed 20 vigorous Single-comb White Leghorn chicks in each of a number of pens and restricted one lot entirely to the basal ration already referred to, which is very low in mineral constituents.[3] All 20 chicks survived and made reasonably good growth, though not so good as the chicks

[2] Reference is made by number (italic) to "Literature Cited," p. 198.

[3] The ration received by lot 828 contained 2.64 per cent total ash material, of which 0.244 per cent was CaO, 1.132 per cent was P_2O_5, and 0.1663 per cent was Mg.

in a parallel pen receiving 2 per cent of tricalcium phosphate in addition. The growth curves for lots 828 and 829 in Figure 1 and of lots 845 and 846 in Figure 2 indicate the beneficial effect of adding 2 per cent of $Ca_3 (PO_4)_2$ to this particular base. Curves 828 and 845, however, demonstrate the ability of the chick to exist on a very low plane of calcium intake for at least eight weeks provided conditions for assimilation and fixation are favorable.

OPTIMUM ADDITIONS OF TRICALCIUM PHOSPHATE

When it was observed that 2 per cent of tricalcium phosphate added to the basal ration had a beneficial effect on the growth rate of chicks, it was decided to add increasing amounts to determine if possible the optium point with respect to these additions. The growth curves for lots 846, 847, and 848, as given in Figure 2, indicate

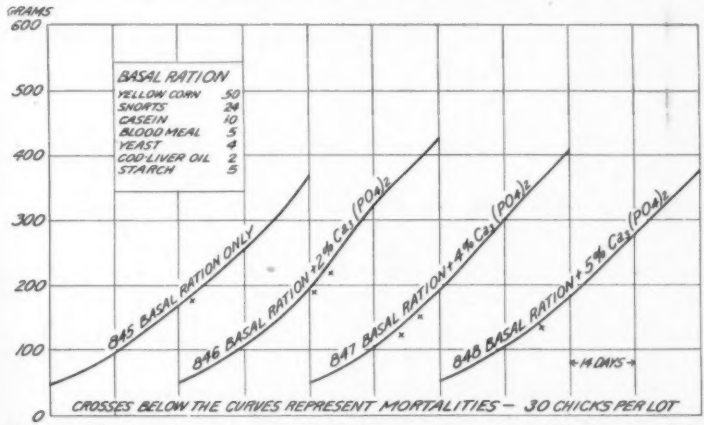

FIGURE 2.—The effect of increasing amounts of calcium upon the growth of chicks, when supplied in the form of the phosphate

no advantage from the addition of more than 2 per cent. The average growth rate of chicks receiving 4 and 5 per cent, respectively, of $Ca_2 (PO_4)_3$ was not so good as for lot 846 receiving only 2 per cent. This is in agreement with experimental work previously reported (*5*) which indicated an excess of mineral elements to be undesirable.

THE EFFECT OF ACID-BASE CHANGES ON MINERAL METABOLISM

Changes in the acid-base values of the ration are believed to influence the assimilation and fixation of calcium and phosphorus.

Shohl, Bennett, and Weed (*7*) observe from the analyses of the bones of experimental animals that the ash deposition is greatest with neutral diets, smaller with alkaline diets, and least with acid diets. Metabolism studies also indicated the largest retentions of calcium and phosphorus on neutral diets. Zucker, Johnson, and Barnett (*10*) report the production of rickets in rats on a diet containing an excess of base over acid. When they substituted $CaCl_2$ for Ca lactate in equivalent amounts they increased the acidity of the diet. The bones of the experimental animals on the latter ration were nearly

normal or showed only mild rickets. The addition of 2 per cent NH_4 Cl, they stated, prevented the development of rickets. They conclude that a diet, which with respect to balance between calcium and phosphorus should not lead to rickets, may do so when there is brought about a lessening of the acidity of the intestinal tract. Jones (*3*), using dogs as experimental animals, concludes that normal animals can well tolerate a certain excess of alkali in diets both high and low in phosphorus, but if the amount is sufficiently increased resorption of bone material occurs. Neutralization of the excess of alkali again initiates healing of the rachitic bone tissue.

Just how much tolerance the chick may have for variations of the acid-base content seems to be a matter of considerable importance, and experiments were planned to obtain information on this point. Figure 3 shows the effect of 2 per cent additions of NH_4Cl, $NaHCO_3$,

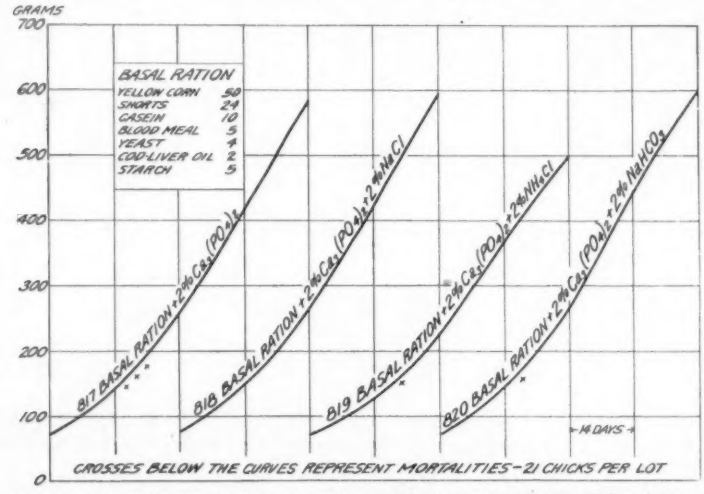

FIGURE 3.—The effect of a tricalcium phosphate supplement upon the growth of chicks, when supplied alone and in combination with various other minerals

and NaCl. With the basal ration used, 2 per cent additions of $NaHCO_3$ and 2 per cent of NaCl were evidently tolerated with no appreciable effect on the growth rate or well-being of the chicks. The addition of 2 per cent of NH_4Cl, however, had a very definite inhibitory effect on the growth rate, though the mortality rate was very low even on this ration, as shown by curve No. 819.

The curves for lots 831 and 832 in Figure 1 show that 4 per cent additions of the acid and base factors referred to proved very disastrous. The mortality rate of the chicks on the alkaline ration was especially high, but the growth rate of the survivors was somewhat higher than that of the chicks on the strongly acid ration.

Figure 4 shows an almost perfect repetition of the results reported in Figure 3, curves 817, 819, and 820, with a large number of chicks on each ration. Apparently with this particular basal ration the alkalinity contributed by 2 per cent of $NaHCO_3$ was tolerated better than was the acidity resulting from the addition of 2 per cent of NH_4Cl.

THE RELATIVE AVAILABILITY OF CALCIUM SALTS

Growth curves for lots 828 and 829 (fig. 1) and for lots 845 and 846 (fig. 2) show that the basal ration used could be improved by the addition of calcium in the form of tricalcium phosphate. An interesting question presents itself: Is there a difference in the availability of calcium in the various forms in which it is most easily furnished? Using rats as the experimental animals, Steenbock, Hart, Sell, and Jones (*8*) added calcium lactate, calcium carbonate, calcium phosphate, calcium sulphate, and calcium silicate at approximately the same plane to the same basal ration. They concluded that no difference in availability of these particular salts exists when they are fed in liberal amounts.

Since rats and birds are known to have somewhat different nutritional requirements, the writers have undertaken to obtain information on the utilization of various calcium salts by the growing chick. Tricalcium phosphate, calcium lactate, calcium sulphate, calcium chloride, and calcium carbonate were compared at exactly the same calcium plane, this being the amount of each of the salts needed to contribute 1 per cent of calcium to the ration. To the ration of one lot (872) was also added 3 per cent of salt mixture No. 185 (McCollum)[4] to determine whether the growth rate could be improved by adding a complex inorganic mixture. The growth curves shown in Figures 5, 6, and 7 indicate that, when added at the same plane, the tricalcium phosphate was better utilized than were the other salts. Calcium carbonate and calcium lactate were quite well utilized, but calcium sulphate and calcium chloride had a decidedly inhibitory effect on the growth rate. Whether this was due to a change in the reaction of the ration on addition of the latter salts must remain a matter of speculation. One can from this evidence conclude that there is a difference in the utilization of the different forms of calcium, and that tricalcium phosphate, calcium carbonate, and calcium lactate are most readily assimilated and fixed.

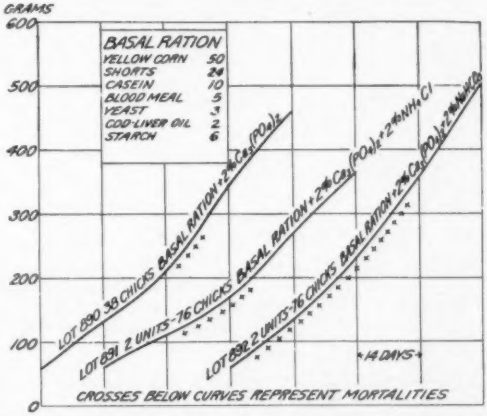

FIGURE 4.—The effect of a tricalcium phosphate supplement upon the growth of chicks when supplied alone and in combination with other minerals

DOES MAGNESIUM INHIBIT CALCIFICATION?

One finds in the literature on calcium metabolism the suggestion that magnesium salts interfere with calcium fixation. Many of the limestones used for poultry feeding contain appreciable amounts of

[4] Salt mixture No. 185 contains the following ingredients expressed in terms of percentage: NaCl, 4.6; $MgSO_4$, 7.1; $NaH_2PO_4.H_2O$, 9.3; K_2HPO_4, 25.7; $CaH_4(PO_4)_2.H_2O$, 14.6; ferric citrate, 3.1; calcium lactate, 35.1.

magnesium. It is therefore important to know whether the amount of magnesium which may be included when impure limestone is used is really detrimental.

Shelling, Kramer, and Orent (*6*) find that calcification occurs most readily in the absence of magnesium. Haag and Palmer (*1*) state

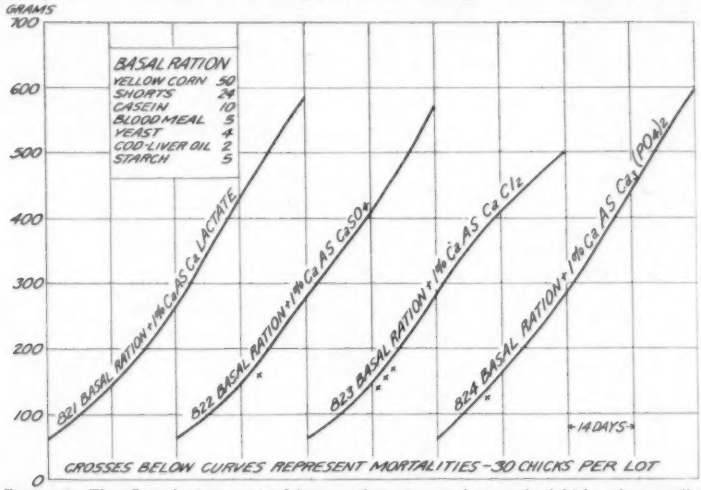

FIGURE 5.—The effect of a 1 per cent calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts

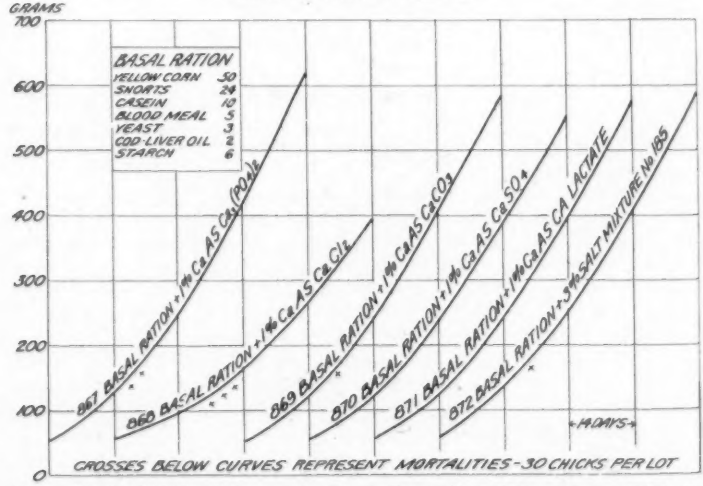

FIGURE 6.—The effect of a calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts, as compared with growth on the basal ration supplemented with a special salt mixture

that a more or less balanced condition of calcium, magnesium, and phosphorus salts of the ration is essential to normal growth and functioning. They found high magnesium content to be a disturbing factor in nutrition. They observed greater tolerance for $MgSO_4$

than for $MgCO_3$ when added in equivalent amounts to the same basal ration. Malcolm (*4*) reports that the ingestion of magnesium salts ($MgCl_2$) may hinder the deposition of calcium in young animals and cause a loss of calcium from the bodies of older animals. Hart

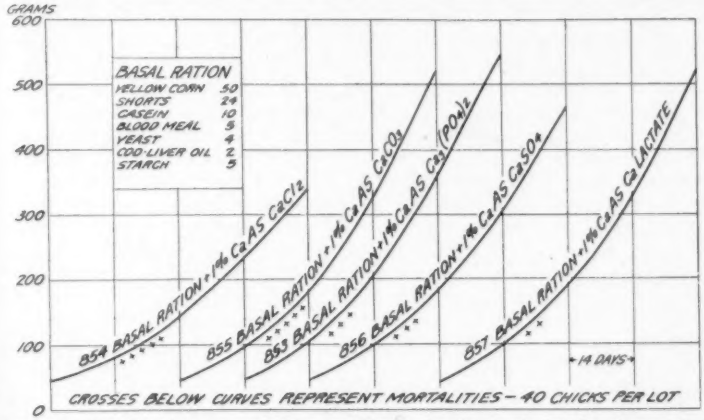

FIGURE 7.—The effect of a 1 per cent calcium supplement upon the growth of chicks, when supplied in the form of various calcium salts

and Steenbock (*2*) found that with pigs the ingestion of excessive amounts of magnesium salts caused an increased excretion of calcium in the urine.

Figure 8 shows the growth history of three lots of 35 chicks each; one lot receiving no magnesium, one receiving $MgCO_3$ in quantity representing 0.5 per cent magnesium, and the third receiving an equivalent amount of $MgSO_4$. That there was some disturbance of the mineral metabolism in lot 864 receiving the $MgCO_3$ was evident from a tendency toward rickets. Seven chicks in this lot were rachitic, whereas the chicks in lot 865 were to all appearances normal.

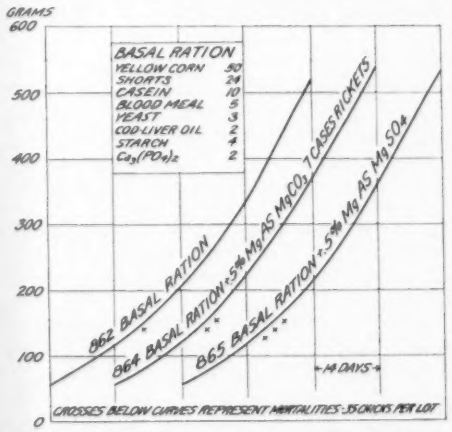

FIGURE 8.—The effect of a 0.5 per cent magnesium supplement, supplied as the carbonate or the sulphate, upon the growth of chicks, as compared with growth on the basal ration containing a tricalcium phosphate supplement

DISCUSSION

The growth period covered by these experiments extended from the second week to the ninth week, inclusive. The weight increase for Single-comb White Leghorn chicks on complete rations during this period is normally about 1,000 per cent of the weight at the beginning of the experiment. Growth during this period should reflect quite

accurately the efficiency with which mineral elements are utilized by the growing chick at a time when the inorganic requirements are relatively high.

The utilization of calcium in normal bone growth appears to depend on two factors, (1) its assimilation, and (2) its fixation in the bone cell itself. The action of vitamin D on calcium and phosphorus assimilation is, according to Yoder (*9*), effective through a lowering of the pH values throughout the intestinal tract. This investigator noted a correlation between lowered pH values and calcium and phosphorus assimilation. Irradiation of rats on a rickets-producing ration likewise resulted in a lowered pH of the intestinal tract, with the distinction, however, that the low pH was observed beyond the duodenum only. This is taken to indicate the excretion of an acid or an acid-producing substance into the small intestine as a result of the action of radiant energy.

In all the experiments for which growth data are here presented the writers have aimed to provide the most favorable conditions for assimilation. As previously noted, a good grade of cod-liver oil was included in the basal ration, and in addition chicks were exposed to direct sunshine. The calcium-phosphorus ratio was not changed when calcium was furnished in each of four forms (not including $Ca_3(PO_4)_2$) and the antirachitic factor was provided. Even with these standard conditions, however, some factor influenced the growth rate in the various lots receiving different forms of calcium. The possibility that calcium in certain combinations may result in faulty mineral metabolism in the bone cell itself is to be considered. Jones (*3*) concludes that the precipitation of bone salts is determined by the balance of ions between the bone-forming cells and their surrounding medium and that such factors as ultra-violet rays, vitamin D, etc., may either directly or by catalysis alter the hydrogen-ion concentration of the bone cells and thus influence the deposition and resorption of bone.

SUMMARY

The growing chick is fortunately able to adjust itself to rather wide ranges of acidity and alkalinity in the diet when vitamin D and ultra-violet radiation are provided. The toleration for acidity and alkalinity is not, however, unlimited, though it probably extends beyond the points which are reached when practical chick rations are fed under farm conditions.

With the particular ration used as a base in these experiments calcium furnished as tricalcium phosphate was more efficiently utilized than was an equivalent amount of calcium furnished as the carbonate, lactate, sulphate, or chloride.

The addition of 0.5 per cent of magnesium, offered as carbonate and sulphate, did not appreciably influence the growth rate of chicks, though rickets resulted from the magnesium carbonate additions.

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